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VOL. 73 JULY 1, 1925 No. 2

RELATION OF TRYPTOPHAN TO THYROID ACTIVITY IN THE WHITE RAT

HSI-CHUN CHANG

From the Hull Physiological Laboratory of the University of Chicago

Received for publication November 24, 1924

The fact that thyroxin appears to be a tri-iodo, tri-hydro-derivative of tryptophan has led Harries (1923) to suggest the importance of tryptophan in the activity of the thyroid gland. He believes, among other things, that "exophthalmic goiter is due to excessive absorption of tryptophan from the intestine." This investigation was primarily undertaken to test this theory.

During the progress of this investigation, Cramer (1923) reported his observations on the thyroids of the rat fed on the maize diet. According to Cramer: "There is a very characteristic atrophy of thyroid gland in the maize-fed rats. The alveoli are small, widely separated, and contain little or no colloid. This condition of the thyroid does not occur in vitamin-B deficiency. The adrenals showed a fair load of adrenalin in the medulla. . . . . Therefore this is evidence for the prediction of Hopkins and Willcox (1906) that tryptophan is the mother substance of the thyroid hormone." He stated also that work on effect of excessive supply of tryptophan on thyroid change is in progress, but his further results have not yet been reported, to our knowledge.

MATERIAL AND TECHNIC. 1. White rats were used as the experimental animals. In all the experiments, only members of the same sex were used in each litter. They were carefully attended to, weighed daily or weekly during the period of experimentation, and fed regularly at a definite time once a day. Food and water pans were washed before each feeding; the cages were kept clean. The room temperature was maintained fairly constant.

2. Diets. Six kinds of diet were used. They were: *a*, control diet—bread and milk; *b*, chronic inanition diet—control diet in insufficient amount; *c*, quantitative maintaining diet—milk and flour; the flour was

used instead of bread for accurate weighing; *d*, aciduric diet—control diet plus about 1 gram of lactose per capita; *e*, qualitative inanition diet, i.e., gelatin diet.

a. Formula:

Bacto-gelatin	25 gm.
Potato starch	42 gm.
Lactose	21 gm.
Butter fat (casein-free)	15 gm.
Salts (McCollum no. 185) (1922)	5 gm.
Water	600 cc.

b. Preparation: The water was warmed to dissolve the gelatin before adding the other ingredients. Constant stirring was applied while the mixture was allowed to solidify in an ice chest.

f, Gelatin diet plus vitamin-B tablets. To each ration of the gelatin diet, five Harris vitamin-B tablets were added. These tablets had been found to give negative biuret.

3. Tryptophan. The tryptophan (Pfanstiehl) was used in 1 per cent solution for injection; 20 or 50 cc. were prepared each time with fresh sterile distilled water in a sterile volumetric flask, and kept at room temperature. The last portion of each preparation was tested for tryptophan by Kraus (1924) vanillin-HCL reagent, and for sterility with agar tube. The tryptophan was found fairly stable at room temperature, but the agar tubes showed slight growth. This was expected because the tryptophan itself was not sterile, and the frequent exposure to air at injections might be another source of contamination. Judging from the local and general condition of the injected animals, however, we feel satisfied about its use. The site of the injection was on the back. A small area was shaved and sterilized with 70 per cent alcohol. The syringe was boiled in water, and the needle immerged in alcohol for ten minutes before using. The injection was done slowly to avoid local overdistention.

4. Tissue preparation. The balance was set ready for weighing. Then care was taken to remove promptly the thyroids, with parathyroids intact, in a bloodless field. The animal was etherized, and after the exposure of the field, the anesthesia was deepened. When the animal ceased breathing, the thyroid-parathyroid glands were quickly removed, care being taken not to touch the glands by the instruments. The glands were dropped into Ringer solution in a small covered dish of aluminum. The whole outfit including the glands, the Ringer, and the covered dish weighed not over 0.9 gram in all the experiments, so that error in weighing was small. A second weighing was taken after transfering the glands into Zenker to be fixed. It took about 5 minutes to finish the whole process. The glands were subsequently imbedded in paraffin, cut serially, and stained with haematoxylin-Von Gieson or eosin in some experiments. The height of the

follicular epithelium was measured with an ocular micrometer and 1.8 mm. objective.

5. Urine. Fresh drops of urine were collected under excitement by rough handling. Obermayer's test was used for indican.

RESULTS. *A. Group I.* This group of experiments was conducted to determine the effect of excessive amounts of tryptophan upon the thyroids.

Five litters of animals were used, three were fed with control diet, two kept on aciduric diet. From 10 to 20 mgm. of tryptophan were injected subcutaneously once a day into each of the selected animals. At least one animal in each litter was used as a control. The weight of the animals was recorded weekly, the room temperature daily.

Tables 1 and 2 summarize the data of four experiments in this group.

TABLE 1

Animals on control diet

AGE AT BEGINNING OF EXPERIMENT IN WEEKS	SEX	DURATION OF EXPERIMENT IN DAYS	TOTAL AMOUNT OF TRYPTOPHAN INJECTED IN GRAMS	INJECTED ANIMALS			CONTROL ANIMALS		
				Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams
				Initial	Final		Initial	Final	
6	♀	111	0.7536	28	130	0.0249	24	120	0.0256
				30	134	0.0201			
9	♂	22	0.4050	132	182	0.0334	130	180	0.0300

TABLE 2

Animals on aciduric diet

AGE AT BEGINNING OF EXPERIMENT IN WEEKS	SEX	DURATION OF EXPERIMENT IN DAYS	TOTAL AMOUNT OF TRYPTOPHAN INJECTED IN GRAMS	INJECTED ANIMALS			CONTROL ANIMALS		
				Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams
				Initial	Final		Initial	Final	
5	♀	42	0.640	53	186	0.0255	38	168	0.0301
							44	160	0.0273
5	♀	47	0.617	31	128	0.0207	28	124	0.0263

In all the experiments of this group, the gross results were negative, and the morphological pictures of the thyroids of the injected animals, moreover, showed no demonstrable difference from those of the controls.

The urine of the animals kept on aciduric diet was not free from indican either in the tryptophan injected or in the control animals, suggesting that the amount of lactose used might not have been sufficient to completely change the intestinal flora.

B. Group II. This group, being represented by one experiment, was a

further study of the effect of excessive amount of tryptophan on the thyroids with a particular purpose of ascertaining whether that effect, if there is any, is produced by the nutritional or specific action of tryptophan.

Preliminary to the experiment, it had been found by a two weeks' test that 3 grams of flour and 5.5 cc. of whole milk per capita per day could maintain the body weight of the animals in this litter fairly constant. On this diet, one of the animals was given from 10 to 20 mgm. of tryptophan daily; the second served as a control (basic control); and to the third animal free amount of control diet was given (normal control). Body weights and room temperature were daily recorded.

Table 3 presents the essential data of this experiment.

In the gland of the basic control, the follicles were numerous, small, oval or spheroidal, loaded with a fair amount of colloid material. Some peripheral follicles were rather large. The epithelial cells were flat, varying from 3.6μ to 5.4μ; their basal membrane was indistinct, or cytoplasm had often so disintegrated that only nuclei remained conspicuous. The fibrous stroma was considerably increased.

TABLE 3

AGE AT BEGINNING OF EXPERIMENT IN WEEKS	SEX	DURATION OF EXPERIMENT IN DAYS	TOTAL AMOUNT OF TRYPTOPHAN INJECTED IN GRAMS	INJECTED ANIMAL			BASIC CONTROL			NORMAL CONTROL		
				Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams
				Initial	Final		Initial	Final		Initial	Final	
5	♀	54	0.295	54	60	0.0211	48	62	0.0219	36	128	0.034

The glands of the animal injected with tryptophan showed a similar picture; the epithelial cells measured from 3.6μ to 5.4μ, 7.2μ being occasionally found.

In the gland of the normal control, however, the follicles were, on the whole, very large and full of colloid; the epithelial cells were well outlined, cubical or columnar, measuring from 5.4μ to 12.6μ; they were richly surrounded by capillaries, and in places the red cells were found protruding into the cytoplasm, or lining the periphery, but presenting such an unique base-line that they might be overlooked under low magnification

Figure 1 illustrates the maximum, minimum and average height of the epithelial cells of typical follicles in the glands of the three animals.

C. Group III. This group of experiments was designed to compare the thyroids of animals on the gelatin diet with those of animals under chronic starvation

Six litters were used in this group. In one litter of five animals, three were put on the gelatin diet, one served as a normal control, and the other

was underfed with the control diet in order to keep its body weight at about the same level as those of the animals on the gelatin diet (weight control). Of the three animals fed with the gelatin diet, one received daily injections of 20 mgm. of tryptophan, the second received five vitamin-B tablets within 5 or 6 days, and the third remained as a gelatin-diet control. Body weights and room temperature were recorded daily.

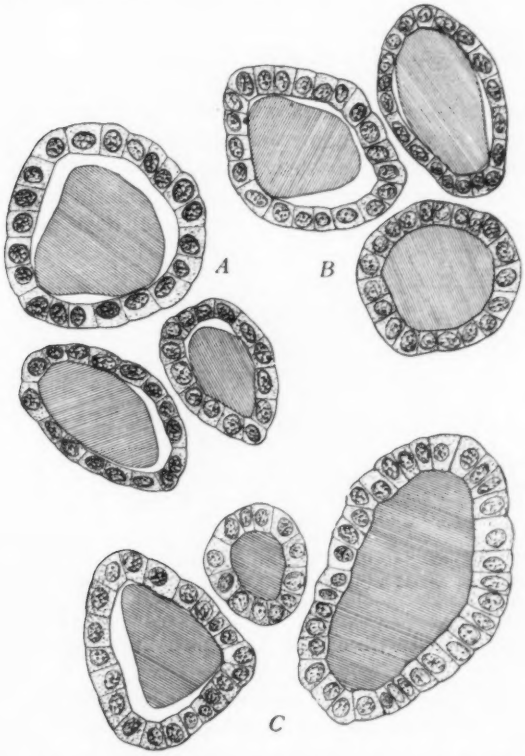

Fig. 1. Camera lucida drawings; magnification 750. *A*, follicles from thyroid glands of rat on normal diet injected with tryptophan. *B*, follicles from thyroid glands of rat on basic control diet. *C*, follicles from thyroid glands of rat on normal control diet.

In litters of smaller size, only part of the experiment as outlined above could be carried out. That is, one was put on gelatin diet, the second on chronic starvation (weight control), and the third served as a normal control.

Table 4 shows the results of two typical experiments of this group.

Attention is invited to the fact that the body weight of the injected animal was maintained while that of the rest sustained great loss except that of the normal control.

In the experiment A, the glands of the animal kept on gelatin diet contained many small follicles, crowded together, with some larger ones on the periphery, being lined with epithelial cells from 3.6μ to 5.4μ in height with reduction in cytoplasm, and slight increase of connective tissue, and containing a fair amount of colloid.

The glands of the animal under chronic starvation presented an identical picture.

In the glands of the animal kept on gelatin diet plus vitamin-B tablets, the follicles were very small, numerous, closely packed; the epithelial cells were from 3.6μ to 5.4μ in height, 5.4μ being the most common.

The follicles of the glands of the animal fed on gelatin diet, but receiving 0.549 gram of tryptophan subcutaneously for 31 days, were moderately

TABLE 4

EXPERIMENT	AGE AT BEGINNING OF EXPERIMENT IN WEEKS	SEX	DURATION OF EXPERIMENT IN DAYS	TOTAL AMOUNT OF TRYPTOPHAN INJECTED IN GRAMS	ANIMALS ON GELATIN DIET PLUS TRYPTOPHAN INJECTION			ANIMALS ON GELATIN DIET PLUS VITAMIN-B			ANIMALS AS GELATIN DIET CONTROLS			ANIMALS AS WEIGHT CONTROLS			ANIMALS AS NORMAL CONTROLS		
					Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams	Body weight in grams		Weight of glands in grams
					Initial	Final		Initial	Final		Initial	Final		Initial	Final		Initial	Final	
A	10	♀	31	0.549	84	84	0.0237	72	62	0.0181	78	57	0.0100	70	62	0.0168	126	152	0.0336
B	5	♂	22	0.276	54	52	0.0180				54	46	0.0109	54	64	0.0132	72	96	0.0217

large, and fully loaded with colloid; the epithelium was from 3.6μ to 9.0μ in height.

The glands of the animal on free amount of control diet, the so called normal control, were very vascular; the follicles large, widely separated, with many capillaries in between; the epithelial cells measured from 7.2μ to 10.8μ, enclosing a considerable amount of colloid. In none of the preceding four glands was the rich vascularity observed.

Figure 2 illustrates the difference in the height of the epithelial cells in these five glands. Three typical follicles of each gland are shown, representing the maximum, minimum and the average height of the epithelium.

In experiment B, the glands of the animal receiving 0.276 gram of tryptophan in 22 days did not show any considerable difference from those of the animals used as the gelatin diet and the weight controls. It was not considered as a negative result because the amount of tryptophan given

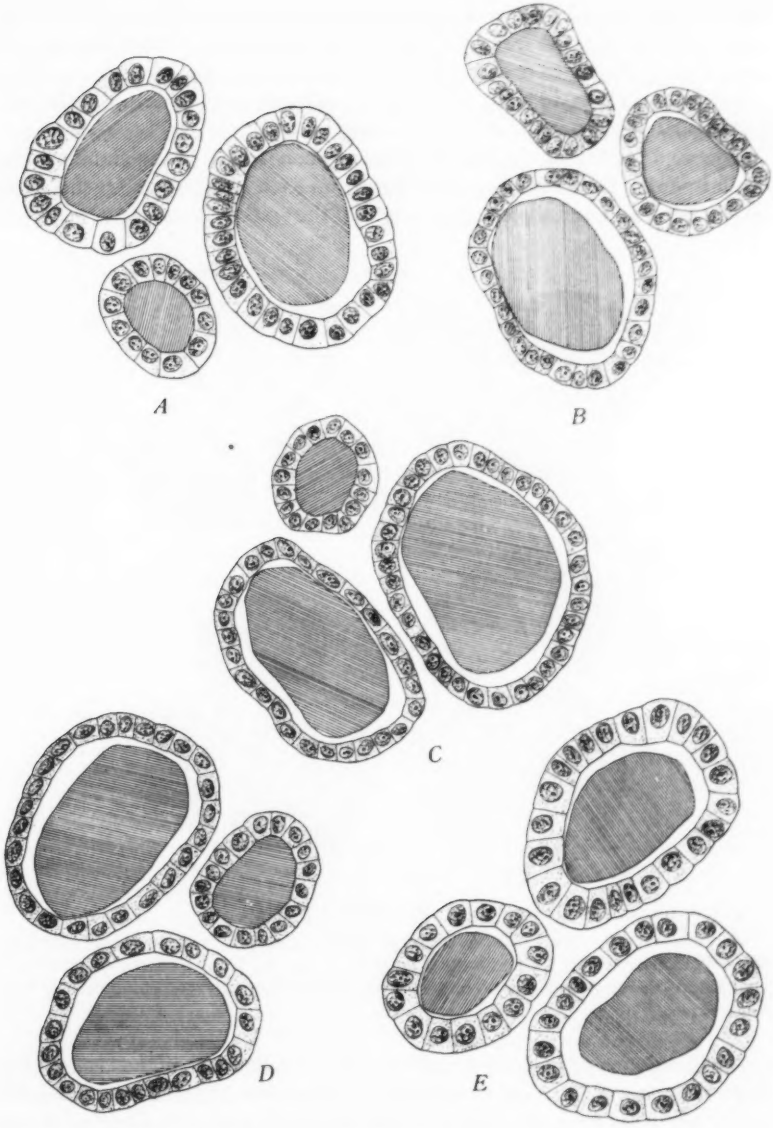

Fig. 2. Camera lucida drawings; magnification 500.

A, follicles from the thyroid glands of rat on gelatin diet plus injection of tryptophan. *B*, follicles from thyroid glands of rat on gelatin diet plus vitamin-B tablets. *C*, follicles from thyroid glands of rat on gelatin diet alone. *D*, follicles from thyroid glands of rat on chronic starvation. *E*, follicles from thyroid glands of rat on normal control diet.

was less and duration of experimentation shorter than those of the experiment A, although the weight of the injected animal had been maintained fairly well. Furthermore, the slight difference of the final weights of these three animals was, in most probability, not sufficient to bring forth morphological differences of the thyroid glands.

Figure 3 illustrates the height of the epithelial cells of the glands of this experiment. Three follicles are taken from each gland showing the maximum, minimum and the average height of the epithelium respectively.

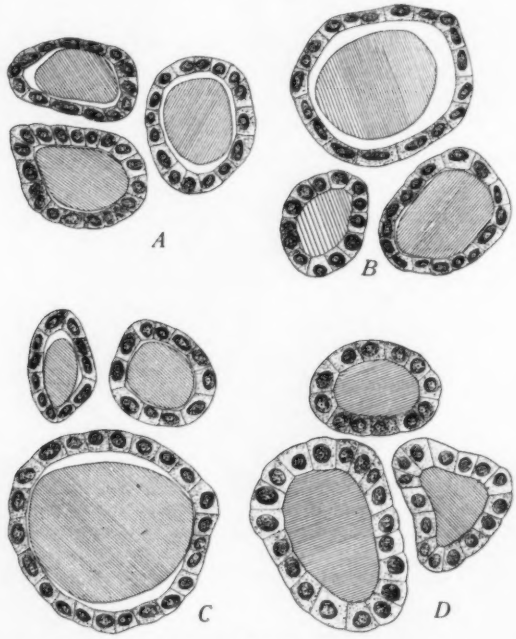

Fig. 3. Camera lucida drawings; magnification 750.

A, follicles from thyroid glands of rat on gelatin diet plus injection of tryptophan. *B*, follicles from thyroid glands of rat on gelatin diet control. *C*, follicles from thyroid glands of rat on basic control diet. *D*, follicles from thyroid glands of the normal control.

D. Correlation of the body weights and the weights of the glands. Judging from the experimental data presented in the tables 1, 2, 3 and 4, a definite relationship between the weight of the body and that of the glands seemed to exist. In order to bring this out more clearly, the miscellaneous data of all experiments carried out in this investigation were used to plot two curves, one for females and one for males, as shown in figure 4. The weight

of the body is indicated on the abscissa, and that of the glands on the ordinate.

The curve (fig. 4) representing the data from the female animals is "positively accelerated" almost throughout the entire length. This means that the weight of the glands is directly proportional to that of the body. The same is true for the curve representing the data from the male animals with body weight up to 156 grams; the later part of the curve, however, shows a "negative acceleration," i.e., the weight of the glands is inversely

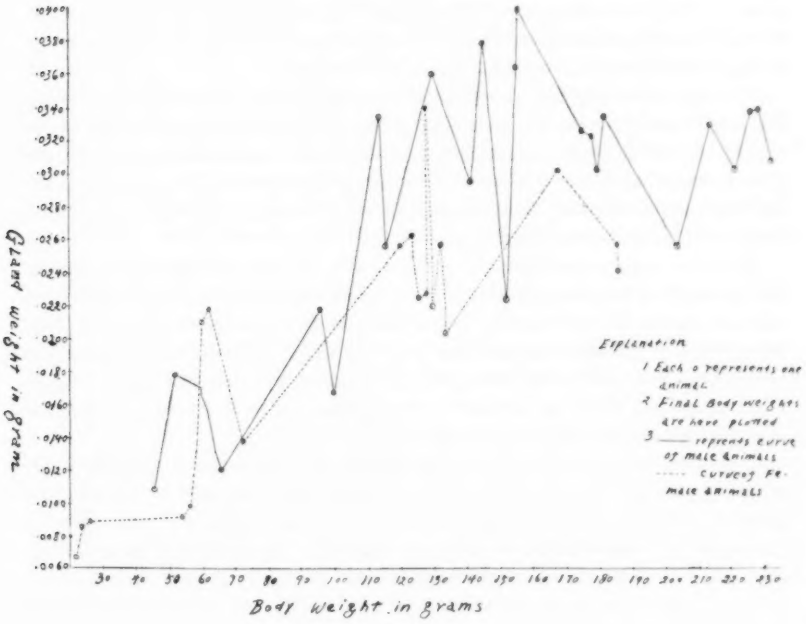
Gland weight in grams
Body weight in grams
Explanation
1 Each o represents one animal
2 Final body weights are here plotted
3 —— represents curve of male animals
------ curve of female animals

Fig. 4

related to that of the body. Since these animals over 160 grams were adults, this inverse relationship may indicate some thyroid atrophy with increasing age.

Discussion. Whether Cramer has enough evidence to support the hypothesis that "tryptophan is the mother substance of the thyroid hormone," we decline to discuss. In our experiments the weight of the thyroid-parathyroid glands, in most cases, varies directly with the body weight which, in turn, varies directly with the nutritional condition of the animal. That tryptophan is necessary for the maintenance of growth is known and is here again confirmed, although additional supply of trypto-

phan in the amount here given (subcutaneously) to the animals fed with adequate protein, with or without lactose, did not further improve their nutritional condition. This means that the substance necessary to maintain the growth may, when it is taken in excessive amount, not be able to accelerate the normal rate of growth. That vitamin-B tablets could not correct the malnutrition of the gelatin-fed animals might, according to the above reasoning, indicate, if the tablets were reliable, that the gelatin diet was not primarily deficient in vitamin-B.

According to Dr. I. Kraus, pure gelatin contains 0.11 per cent of tryptophan. Whatever amount of tryptophan that may be present in the commercial bacto-gelatin used in these experiments, it did not prove to be enough for the nutritional requirement of the rat.

It would seem logical to assume that, within the physiological limits, the larger and heavier the gland, the greater is its activity; and this is indicated by the fact that the height of the follicular epithelium of the larger glands was found to be almost double that of the smaller glands in the different experiments. The amount of colloid is not emphasized because it can not be measured quantitatively.

With our results in mind, we question the specific action of tryptophan on the thyroid hormone, and we are inclined to believe that the atrophic changes in the thyroids of the white rats fed on tryptophan-deficient diet, as noticed by Cramer , can be explained as the result of chronic inanition (Jackson, 1916), only it is here qualitative instead of quantitative. It goes without saying that the present investigation does not seem to support Harries' contention, at least for the rat.

In Jackson's and the present experiments, the decrease in growth of the animal parallels the atrophic change or decreased activity of the thyroid glands. But thyroid deficiency will by itself retard body growth (cretinism). This reciprocal relation between thyroids and growth suggests that good nutrition might first improve the body growth which, then, brings forth more active development of the thyroid glands; or it might first invigorate the thyroids which, in turn, stimulate the body growth. Of the two possibilities, the first seems to be more probable.

SUMMARY

1. In rats on either milk-bread or milk-bread-lactose diet, subcutaneous injections of tryptophan, ranging from 0.4 gram to 0.7 gram during periods as short as 22 days, and as long as 111 days, did not produce any demonstrable morphological changes of the thyroid glands deviating from those of the controls.

2. On the gelatin diet, the animals lost weight and developed eventually the condition of marasmus. In these animals, a "hypo-" condition of the

thyroids was present as judged mainly by the decreased height of the secreting cells and the decreased weight of the glands. The animal in chronic inanition with body weight maintained at the same level as that of the gelatin-fed did show, however, a similar thyroid picture.

3. Vitamin-B tablets in the amount given could not correct the malnutrition of the animals on the gelatin diet.

4. Animals on the gelatin diet given subcutaneously 0.549 gram of tryptophan for a period of 31 days did show an improved nutritive condition, maintaining the body weight. The thyroids of the injected animals, moreover, appeared practically normal.

5. With the body weight maintained fairly constant by the quantitative maintaining diet, i.e., 3 grams of flour and 5.5 cc. of whole milk per capita per day, 0.295 gram of tryptophan injected subcutaneously for a period of 54 days did not bring up the body weight of the animal more than that of the control, indicating that tryptophan can not accelerate the rate of growth in animals on adequate protein although in limited amount.

6. The weight of the thyroids with parathyroids intact was found, in most cases, to be directly proportional to the body weight irrespective of the different diets.

7. Jackson's results showing thyroid atrophy or degeneration from chronic starvation is confirmed.

8. Harries' contention does not hold in the white rats, because the animals receiving excessive amounts of tryptophan were morphologically normal.

9. Cramer's results are partially confirmed. We can not accept his interpretation, while lack of tryptophan in the diet may directly affect the thyroid by lack of material for production of the thyroid hormone; we are more inclined to believe that the body weight of the animal, i.e., the general nutritional condition, is the important factor responsible for the thyroid changes, since these appear in parallel states of malnutrition not due to lack of tryptophan.

The writer wishes to thank Professor Carlson for his valuable criticism, suggestions and help in making this investigation possible. He is also indebted to Mr. C. Tsai for suggestions and repeated supply of experimental animals, and Mr. C. Y. Chang for his preparation of the illustrations, suggestions and help in microscopic technic.

ADDENDUM. When this paper was ready for the press, Hammett's work (1924) on the correlation of the body weight and weight of thyroids of normal rats appeared This, in a sense, is a study of normal variation of the body weight and weight of thyroids under strictly controlled condition.

Concerning the explanation of the positive correlation of the weight of thyroid and body weight, we do not agree with Hammett. He emphasizes the thyroid as a cause

of the increased body growth ("The heavier body is heavier because of heavier and more efficient thyroid"). On the basis of our experimental results, however, it seems more probable to us that the nutritional condition of the animals determines the weight and efficiency of the thyroids, since the thyroids are decreased in weight and appear less active morphologically when the weight of the animals is reduced by qualitative or quantitative starvation.

BIBLIOGRAPHY

CRAMER. 1923. Journ. Physiol., lvii, lxix.
HAMMETT. 1924. This Journal, lxix, 510.
HARRIES. 1923. Brit. Med. Journ., i, 553.
HOPKINS AND WILLCOX. 1906. Journ. Physiol., xxv, 88.
JACKSON, 1916. Amer. Journ. Anat., xix, 305.
KRAUS. 1924. In press.
MCCOLLUM. 1922. Journ. Biol. Chem., liii, 313.

TRYPTOPHAN AND THYROID FUNCTION

A. R. ABEL, R. W. BACKUS, H. BOURQUIN AND R. W. GERARD

From the Department of Physiology of the University of South Dakota

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It has long been known that it is impossible to maintain an animal on a diet inadequate in tryptophan. Though the mode of metabolism of this amino acid is obscure (Lusk, 1919) we do know that it is a constituent of practically every protein thus far analyzed, and the similarity between its structural formula and that of Kendall's thyroxin suggests that it may be essential in the elaboration of the secretion of the thyroid gland.

METHODS. White rats bred from the Tyler and Experimental Colony strains of the Wistar Institute were used in the work. The rats were raised on a stock diet of bread, corn, milk, green vegetables and wheat germ until they weighed about 60 grams, when each litter was divided into four groups and one group put on each of the four experimental diets. In a few cases rats were lighter or heavier at the time the special diet was begun.

The rats on the special diet were kept in individual cages made of galvanized iron screen with a screen bottom. These cages were cleaned systematically.

Record of the age, weight, general appearance and behavior of each rat was made every two days until the animal died or was killed. A careful autopsy was performed in almost every case and tissues were taken for histological study. These tissues were fixed in Bensley's solution, embedded in paraffin, sectioned at a thickness of 10 mirons and stained with hematoxylin and orange G. The weights of all such organs were also recorded.

Diets: These were planned to show the effects of tryptophan lack, and the extent to which these effects are relieved by thyroxin.

1. Tryptophan-free. This diet contained gelatin, 15 per cent; zein, 10 per cent; cornstarch, 50 per cent; yeast extract (in terms of dried yeast), 4 per cent; filtered butter, 7.5 per cent; cod liver oil, 5 per cent; salt mixture, 5 per cent; and agar agar, 3.5 per cent. The zein was prepared from a commercial corn gluten by alcohol extraction and saline precipitation; the yeast extract was made according to Miller (1921); and the salt mixture was the one used by Osborn and Mendel (1917). All materials were tested and found tryptophan free. The diet was prepared by dissolving the agar and gelatin in water and stirring in a mixture of the other ingredients.

2. Tryptophan-free plus tryptophan-control. This is a theoretically perfect diet made from diet 1 by adding 0.33 mgm. of tryptophan a day per gram weight. The amount present in 7.5 grams of an 18 per cent casein diet is 20 mgm., which represents the average daily intake of a 60 gram rat. The tryptophan was prepared by the Special Chemicals Company according to S. W. Cole. All the tryptophan was added to a small amount of diet 1 each day just before feeding, the remainder of the day's ration being added after this had been eaten.

TABLE 1

GRAMS* PER 100 GRAMS TOTAL DIET	18 PER CENT CASEIN DIET	10 PER CENT ZEIN, 15 PER CENT GELATIN DIET
Glycine	0.08	2.48
Alanine	0.34	1.10
Valine	1.44	0.34
Leucine	1.75	2.26
Proline	1.37	1.68
Phenylalanine	0.70	0.71
Aspartic acid	0.32	1.01
Glutaminic acid	3.92	2.90
Serine	0.09	0.16
Tyrosine	0.81	0.35
Arginine	0.69	1.29
Histidine	0.45	0.10
Ammonia	0.29	0.36
Cystine	0.013†	?
Lysine	1.372	0.413‡
Tryptophan	0.27	0.000
Per cent of protein accounted for	77.11%	57.72%

* Calculated from analyses quoted in Matthews' *Physiological Chemistry*, 3rd ed., p. 129.

† A 9 per cent casein diet is made inadequate in cystine, so 0.01 gram per 100 grams of diet may be considered the minimum of complete adequacy.

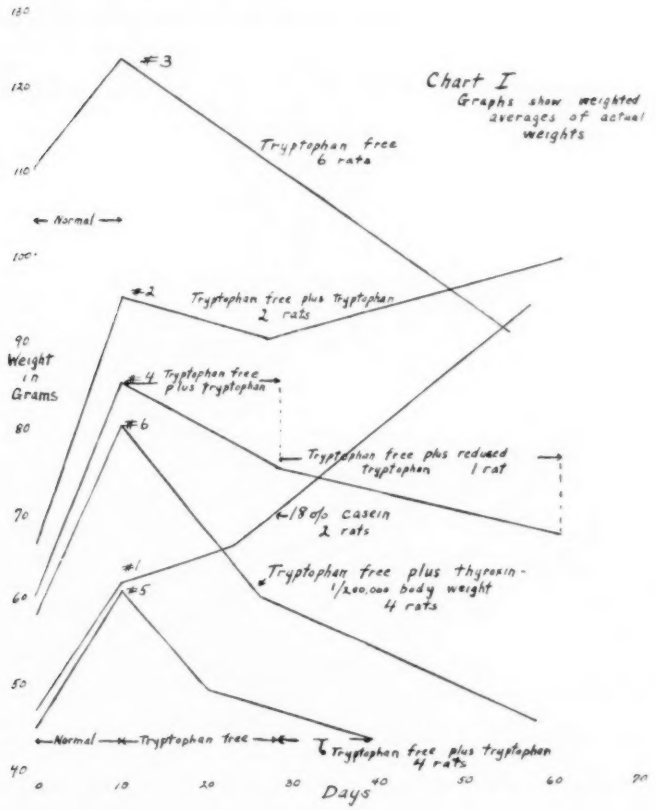
‡ Edestin contains 1.65 per cent lysine, and 18 per cent of this protein is entirely adequate. Such a diet contains only 0.3 gram lysine per 100 grams of diet.

3. Casein control. Eighteen per cent casein replaced both proteins of diet 1 in a few cases; in the majority, by mistake, 10 per cent casein was substituted for 10 per cent zein. Diets 2 and 3 served to check the adequacy of all non-protein factors of diet 1.

4. Tryptophan-free plus thyroxin. To diet 1 pure thyroxin (Squibb & Co., crystals) was added in quantity equal to 1/100,000 or 1/200,000 of the body weight of the rat being fed; about 0.5 or 0.25 mgm. respectively. This dosage, 200 to 500 times the equivalent for humans, was based on the work of Cameron and Carmichael (1921) but it may have been a large

excess. The thyroxin was mixed with a little cornstarch to permit accurate weighing, and added to the diet in the same manner as the trytophan above.

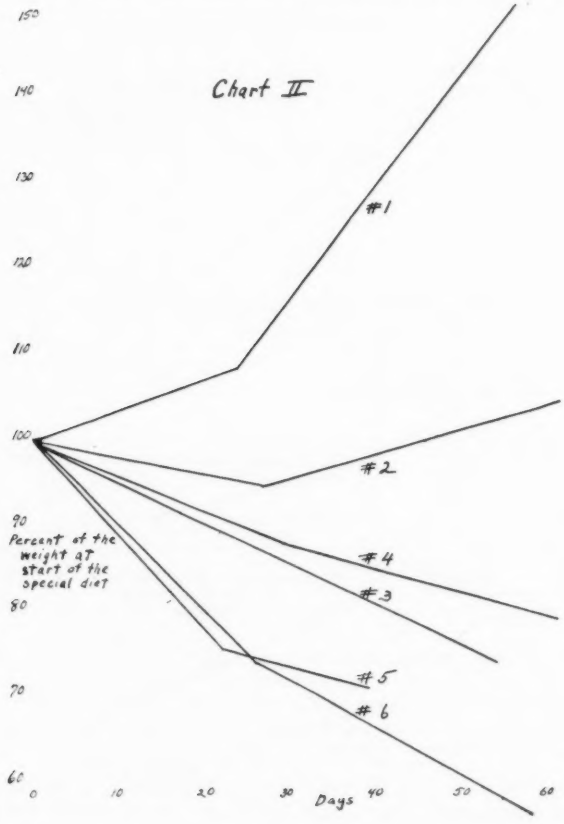
It will be noted that our stock diet 1 is relatively high in protein and low in fat. This was desirable to insure adequacy, though it may have helped



render growth slow. The quantity of each amino acid in this mixture compares favorably with the amount in an 18 per cent casein diet (table 1), cystine alone being possibly deficient. The temperature in the animal room fluctuated rather markedly, which may also have hindered growth. Fortunately these factors obtained for control and test animals alike and can have no bearing on the results noted.

RESULTS. The results obtained can be most easily recorded according to diets.

1. Casein diet: The two rats on the 18 per cent casein diet made an average daily gain of 1.9 grams till they reached the weights of 121 grams and 100 grams, when they were killed and autopsied. Two of the rats on the 10 per cent casein diet made the same gain, the other three gaining at



the rate of 0.9 per cent of the total body weight, or ½ gram daily (table 2, charts 1 and 2). All of these rats appeared normal in every respect and on autopsy all the organs appeared normal.

Histologically the thyroids of these rats, and those of five others transferred from a tryptophan-free to a casein diet for periods of 23 to 52 days before being killed, corresponded with Shafer's (1924) description of a nor-

mal gland, the epithelium of the alveolus being low cuboidal in form with luminal surfaces often rounded and the alveoli small except peripherally where they were more distended and the epithelial cells were more flattened.

2. Tryptophan-free plus tryptophan diet: These rats, fifteen in number, showed no weakness but were stunted in growth. Although they appeared healthy, ate well, and grew decidedly in body length, they did not show a corresponding increase in body weight, remaining at about a constant level

TABLE 2

DIET	TOTAL NUMBER RUN	NUMBER OF THYROIDS EXAMINED HISTOLOGICALLY	AVERAGE PER CENT OF TOTAL BODY WEIGHT FORMED BY THYROID	AVERAGE DAILY INCREASE IN LENGTH	NUMBER OF RATS AVERAGED FOR LENGTH	AVERAGE DAILY CHANGE IN WEIGHT	NUMBER OF RATS AVERAGED FOR WEIGHT	AVERAGE DURATION OF FEEDING SPECIAL DIET
				cm.		*grams*		*days*
Stock	7	7						120
Casein	7	6				+0.90	2	35
Tryptophan-free and tryptophan	15	11	0.015	0.067	1	+0.06	2	30
Tryptophan-free	17	8	0.018	0.06	3	−0.69	10	42
Tryptophan-free, later tryptophan added	12	7	0.015	0.03	3			20—first 20—second
Tryptophan-free, later thyroxin added	10	8	0.012	0.045	9	−1.4	2	26—first 34—second
Tryptophan-free and thyroxin	10	10	0.010			−0.37	2	40
Tryptophan-free later casein diet	4	3						28—first 40—second
Total	82	60						

(table 2, charts 1 and 2). Upon autopsy no disorders were found, and the histological picture of the thyroid was identical with that of the casein diet, indicating that they were normal.

With two of the rats in this group an interesting variation was made by reducing the amount of tryptophan to 1/200,000 of the body weight, about 1/100 of the previous allotment. The result was a gradual loss in weight, with finally the appearance of the tryptophan-free symptoms described below. Upon histological examination of the thyroid, however, the organ was found apparently normal.

3. Tryptophan-free: In this group of seventeen rats a fairly definite clinical picture developed in one to six weeks, depending seemingly upon the weight at the beginning of the diet. The symptoms included a progressive loss of weight, with gradually increasing nervous tremor of the limbs; poor coördination of movements, though with perfect sense of equilibrium; the development of a hump in the back; hair ragged, and falling out to some extent; head broadened disproportionately (probably the general emaciation made this more apparent than real, though it might have been due to skeletal or skin changes); sexual characteristics distinctly affected, as indicated by delay of the testes in descending. If allowed to remain in this condition for any length of time, the rats suffered a severe anorexia, generally dying in convulsions in three to five days. Increasing the amount of vitamin in the diet produced no alleviation of the symptoms. It is noteworthy that no pot belly developed, that muscular weakness was not marked, and that definite skeletal growth occurred, as was shown by increase in body length (table 2, chart 1 and 2).

Histologically the thyroid showed a fair proportion of distended alveoli with flattened epithelium, but the majority of alveoli were very small with high cuboidal epithelium so that large areas of the gland were more cellular than a normal gland. This increased cellularity was not of a degree found in the typical "hyperplasia" but was distinct as compared to the normal.

4. Tryptophan-free diet, later tryptophan: Twelve rats changed from the tryptophan-free to a tryptophan diet immediately upon development of symptoms, were found to be little relieved in general appearance or in loss of weight, the clinical picture and time of death being practically the same as for those on the tryptophan-free diet (charts 1 and 2 and table 2).

Microscopically the thyroids presented the picture of normal organs.

5. Tryptophan-free, later changed to thyroxin diet: Administration of thyroxin to a rat in the typical condition described seems to hasten the loss of weight (see table 2, charts 1 and 2), in spite of an increases in appetite. Upon autopsy many of these rats were found to have intestinal or pulmonary hemorrhage. Histologically there was a variation in thyroids, some appearing definitely colloidal in type, and others normal. Many glands were colloid at the periphery and normal centrally.

6. Tryptophan-free plus thyroxin diet: Animals changed directly from normal to thyroxin diet became very nervous and emaciated, but maintained a good appetite and general physical condition up to the time they were killed for autopsy. No symptoms of a tryptophan-free diet developed (table 2, charts 1 and 2).

Histologically the thyroids in this group were distinctly colloid with large alveoli throughout surrounded by flattened or scale-like epithelium and

with very little interalveolar tissue to be found anywhere. The average per cent of the total body weight formed by these glands was distinctly lower than that for any other group (table 2). This and the appearance indicate a tendency to atrophy.

DISCUSSION. If the thyroid gland cannot synthesize the indole nucleus and that nucleus is essential to the formation of its secretion, as work on thyroxin indicates, the only source for it would be tryptophan. This tryptophan might be available from the diet only or, in the case of a tryptophan-low diet, from the disintegration of tissue proteins. If tryptophan in adequate amounts is available to the gland from that source no signs of hypothyroidism should develop on a tryptophan-free diet unless the gland suffers indirectly from the general breakdown of other organs. If, on the other hand, tryptophan from the latter source is not sufficiently available, evidence of hypothyroidism might result from such a diet and these evidences should be relieved by the administration of tryptophan or thyroxin.

Signs of hypothyroidism in these rats must necessarily be difficult to determine. Hammett, in a personal communication, states that no gross changes occur even in his thyro-parathyroidectomized rats except diminished rate of growth. Here is the further difficulty of the tryptophan free diet which must of itself produce a profound disturbance of metabolism and cell structure (aside from any relation to the thyroid) as it is an important constituent of most, if not all, tissue proteins; and these effects might stimulate or differ from those produced by a condition of hypothyroidism and hence confuse or obscure the latter.

Our results, though not entirely conclusive, indicate that the tissues do yield sufficient tryptophan for thyroid function; not, however, without considerable embarrassment to the gland.

The appearance and behavior of the rats on a tryptophan-free diet were not comparable to the findings in hypothyroidism in man or rabbits, and histological studies, in so far as they went, differed from the changes described by Tatum (1913) in thyroidectomized rabbits. Histologically strain on the gland might be indicated by atrophy, as in inanition (Jackson, 1916), or by hyperplasia, the compensatory reaction of a gland producing a normal or decreased amount of secretion under difficulties, as in iodine deficiency (Marine, 1921, 1922). Sections of the thyroid from the rats on a tryptophan-free diet showed no marked abnormality comparable to the profound general disturbance produced by this diet. They were neither atrophic nor markedly hypertrophic. Finally, addition of thyroxin produced a colloid gland definitely lighter in proportion to total body weight than the glands of animals not receiving it, which suggests that the thyroid had been somewhat active before the administration.

This evidence supports the view that the upset produced by tryptophan

lack involves the body tissues in their entirety, and is largely extrathyroid. This gland appears, in fact, so nearly normal that it seems to obtain the necessary amino acid from the blood plasma at the expense of other tissues and continues to the end to produce its secretion.

On the other hand, some hypo-function of the thyroid, and this under difficulty, is indicated by the following facts. Thyroxin, when fed from the start, prevented the development of the typical tryptophan-free symptoms. Second, the thyroids of rats on tryptophan-free diets, though not showing the classical hyperplasia, did exhibit a definite increased cellularity consistent with moderate strain. Addition of minute quantities of the needed amino acid to the diet, though not relieving the symptoms, kept the glands entirely normal.

Since this work was completed Cramer (1923) has published the results of feeding a diet containing all of the protein in the form of maize meal and therefore tryptophan-low. Rats on this diet developed symptoms apparently similar to those obtained in this laboratory but the thyroids atrophied in a very characteristic manner, the alveoli being small, poor in colloid, and widely separated. Such a condition of the thyroid is commonly believed to occur if the strain on the gland is long-continued and severe, or as a result of marasmus. Cramer has not yet reported the details of his work but it seems certain that on a tryptophan-low diet his experiments would have been continued over a much longer period of time than was possible on a tryptophan-free diet fed to young rats, which died on the average within forty days after being placed upon it. Is that case the two researches are entirely compatible.

SUMMARY

1. White rats were fed on diets similar except for their content of tryptophan, thyroxin, and protein. The variations in the diets were: *1*, protein as casein largely or entirely; *2*, as zein and gelatin with the addition of tryptophan; *3*, as zein and gelatin with no added tryptophan or thyroxin; *4*, as zein and gelatin with the addition of thyroxin.

2. Rats on the tryptophan-free diet developed characteristic symptoms leading to death. The thyroid gland showed a slightly increased cellularity. Minute amounts of tryptophan in the diet did not alter the symptoms but prevented any change in the thyroid. The symptoms once developed were not relieved by later adding tryptophan or thyroxin to the diet though thyroxin transformed some of the glands to the colloid state with evidences of atrophy. Thyroxin added to the tryptophan-free diet from the start rendered the thyroid colloid and very atrophic and prevented entirely the tryptophan-free symptoms, except emaciation and death.

3. These facts indicate that: Tryptophan is essential to the normal functioning of the thyroid; a large amount of the needed tryptophan is available for the thyroid from the tissues of the body; the thyroid is able to take up tryptophan from the blood in quantities approximating its needs, even when the total amount is so small that other tissues suffer from its lack; sufficient thyroxin from an outside source induces atrophy of the thyroid. Lack of tryptophan results also in extrathyroid disturbances leading to emaciation and death.

BIBLIOGRAPHY

CAMMERON, A. T. AND CARMICHAEL. 1921. Journ. Biol. Chem., xlv, 69; Ibid., xlvi.
CRAMER, W. 1923. Journ. Physiol., lvii, Proc. Physiol. Soc.
JACKSON. 1916. Amer. Journ. Anat., xix, 305.
LUSK, G. 1919. The Science of Nutrition.
MARINE, D. 1921. Journ. Amer. Med. Assoc., lxxvii, 14.
1922. Physiol. Rev., ii, 521.
MILLER, E. W. 1921. Journ. Biol. Chem., xlviii, 339.
OSBORNE, T. B. AND L. B. MENDEL. 1916. Journ. Biol. Chem., xxv, 1; 1917 xxxi, 309.
SCHAFER, E. A. 1924. The Endocrine Organs, i.
TATUM, A. L. 1913. Journ. Exper. Med., xvii, 636.

THE EFFECT OF TEMPERATURE UPON THE TONUS OF SKELETAL MUSCLE

F. E. EMERY

From the Physiological Laboratories, University of Illinois

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Studies made upon the variation in the height of the knee-jerk, Lombard (1887), show that in general it is increased and diminished by whatever increases and diminishes the activity of the central nervous system. In one experiment Lombard (1887) showed that cold weather tends to increase and warm weather to decrease the height of this reflex. These observations were made on successive days and were influenced by barometric pressure, humidity and electric changes in the air.

Recently Tuttle (1924 b) and others have given evidence that the increase in the height of the knee-jerk of a subject during increased mental activity, as in solving a problem, is due to increased muscular tonus, and contends that the knee-jerk may be taken as an index to the phenomenon of tone. In this investigation the knee-jerk is used as an index to muscle tonus.

In order to determine the influence of a change in temperature alone on muscle tonus, experiments were carried out in which the temperature was rapidly raised and lowered during the time a continuous record of the knee-jerk was being made. The data obtained from these experiments are discussed in this paper.

The subjects, except the writer (subject E), were all students in athletic coaching, and were accustomed to exercise above that of an average individual. During the experiments the subjects wore only short underwear or, in some instances, only track trousers. These men were all trained in knee-jerk experiments, thus it was not difficult to keep them silent and in a passive attitude.

The data were obtained by an apparatus described by Tuttle (1924 a). The experimental work was done on cold nights in a small room where the temperature could be lowered rapidly by raising the windows, or raised rapidly by closing the windows and turning the steam in the radiators.

The usual procedure was to turn the heat off some hours before the beginning of the experiment in order to cool the radiators and thus not influence the cold period. To insure a more uniform temperature in the room two fans were kept going throughout certain parts of the experiments.

By this arrangement the temperature could be lowered 20° to 40°F. within two to five minutes. A continuous record of the subject's knee-jerk was taken during the temperature changes. The number of stimuli delivered on the ligamentum patellae depended on the number required to secure a characteristic record of the subject's knee-jerk. A subject was considered as giving a characteristic knee-jerk record when he began repeating himself. The subjects were allowed to deliver normal knee-jerks for five or ten minutes at room temperature. Without disturbing them the windows were opened, the result being a rapid decrease in temperature. A five to ten minute record at cold temperature was made. The windows were then closed and the heat turned on, which caused a rapid rise in temperature, reaching 70° to 80°F. within a few minutes. The subjects continued the records five to ten minutes longer at room temperature. By this means a continuous knee-jerk record of each subject was made showing the height of the knee-jerk at room temperature, decreasing temperature, constant cold temperature, increasing temperature, and, in some instances, a second room temperature.

The data are considered in three groups since it was not decided to make a quantitative study when this experiment was begun and the first records show only a relative comparison of the change in muscle tonus with the change in temperature. Furthermore, during the first part of this experiment an electric fan was turned on the subject only during the time he was exposed to cold. This procedure was found to be so effective in distributing the cold that in obtaining the second series of records it was used in this manner throughout.

Under the conditions just set forth, six records were made by two subjects on three different days. The data collected are shown in table 1. In each case the subject was absent from the room during the time the temperature was being changed.

In table 1 a comparison of the height of the knee-jerk is made during: a, normal period at room temperature; b, a cold period; and c, a cold period in which air from a fan is blown continually on the subject.

In consideration of the data presented in table 1 it is evident that with two exceptions (subjects E and B, December 31) a decrease in the temperature caused an increase in the average height of the knee-jerk. In the absence of the fan the greatest increase was 17 mm. obtained from subject E on December 29 and from subject B, January 1. For subject E the height of the knee-jerk increased from an average of 23 to 41 mm. and for subject B from 34 to 51 mm. The minimum increase was 5 mm. obtained from subject B on December 29.

It is also interesting to note the increase in the height of the knee-jerk during the constant cold temperature with air from a fan blowing on the subject. It will be seen, table 1, that the temperature remained about the

same during this period as during the constant cold period without the fan. That the muscle tonus was increased when the fan was on is evident by the increase in the height of the knee-jerk during this period. Upon questioning the subjects they all said they felt much colder when the fan was on. The writer confirmed their statements and found it impossible

TABLE 1

The height of the knee-jerk in millimeters. (A) a normal period at room temperature, (B) a cold period, (C) a cold period with air from an electric fan blowing on the subject

SUBJECT	DATE	(A)		(B)		(C)	
		Temperature	Height of knee-jerk	Temperature	Height of knee-jerk	Temperature	Height of knee-jerk
		°F.		°F.		°F.	
E	December 29	78	23	62 56	41	56	67
B	December 29	78	45	56 52	50	52	73
E	December 31	79	54	52	51	53	67
B	December 31	79	44	56	44	58	68
E	January 1	78	39	57	45	57 54	58
B	January 1	78	34	54	51	54	69

TABLE 2

The height of the knee-jerk in millimeters with air from an electric fan blowing on the subject. (A) a normal period at room temperature, (B) a cold period.

SUBJECT	DATE	(A)		(B)	
		Temperature	Height of knee-jerk	Temperature	Height of knee-jerk
		°F.		°F.	
S	January 13	79	37	44	56
MC	January 21	70	7	53	27
C	January 22	72	2	53	11

to keep from shivering when the fan was on him during the cold period. Without exception, the body tonus, as represented by the height of the knee-jerk, was markedly increased with a decrease in temperature when the electric fan was turned on the subject. In case of subject E, December 29, the height of the knee-jerk is almost three times greater during this

period than during the normal period and over one and a half times greater than during the cold period without the fan. The minimum increase amounts to 13 mm. (table 1, subject E, December 31).

In obtaining the second series of records, (subjects S, MC and C, the same procedure was followed as in the case of subjects E and B, except that the fans were kept running throughout the experiment, one of them being directed on the subject. Table 2 contains the data secured from this group of subjects.

The data in table 2 are not presented on a percentage basis, nevertheless it is interesting to compare subject S with subject MC. Both of these subjects show approximately a 20 mm. increase in the average height of the knee-jerk during the cold period, but the percentage increase would be much greater in the case of subject MC. Also a general survey of the data collected shows that the smaller the height of the normal knee-jerk the greater will be the percentage increase during the cold period. A further study of table 2 shows that without exception the muscle tonus is increased when the temperature is decreased as represented by the height of the knee-jerk. In the cases of MC and C the knee-jerk is approximately four times greater than normal.

A study of the records obtained in this series shows that the height of the knee-jerk increased in a rather uniform manner as the temperature was lowered, until a fairly constant higher kick was established. As the room was again warmed the height of the knee-jerk decreased gradually until a uniform height corresponding to the normal was reached. These observations suggested a more detailed study of the problem and three of the subjects in this series were used to secure more detailed records. The conditions of the experiment were the same as previously discussed except that a signal magnet was used to mark each change of four degrees Fahrenheit. From these records measurements were made of the height of the knee-jerk for every four degrees change in temperature. From these measurements the median (Rugg 1917) was obtained and recorded in table 3.

Subject R is a Junior in college, 19 years old and weighs 180 pounds. He is in good physical condition but not on any of the varsity teams. He is above the average as a student. He is very peculiar in dress and manner but very observing. This subject was the least irritable of all and the blow on the ligamentum patellae had to be increased in order to get a response. The record obtained from this subject, (fig. 1), although not as uniform as the other records of the series, (figs. 2 and 3), shows the usual increase in the height of the knee-jerk during a decreasing temperature.

This subject showed a marked response to the cold by an increase in the height of the knee-jerk from 1 to 16 mm. during the first four degrees drop in temperature (table 3). As the temperature fell the height of the

knee-jerk reached a constant higher level at approximately 64°F. When the temperature was raised, a marked drop in the height of this reflex occurred during the first eight degrees rise in temperature as represented by a height of 19 mm. at 43° to 40°F. as compared to a height of 7 mm. at 44° to 47°F. When the temperature reached 75°F. the height of the knee-jerk corresponded to the normal being only 1 mm. (table 3).

Subject H is a Junior in college, weighs 165 pounds, is 20 years old and in about the same physical condition as subject R. He is very impulsive.

TABLE 3

The median height of the knee-jerk in millimeters for every four degrees change in temperature

TEMPERATURE	MEDIAN HEIGHT OF KNEE-JERK		
	Subject R	Subject H	Subject F
°F.	*mm.*	*mm.*	*mm.*
79–76			27
75–72	1		30
71–68	16	3	34
67–64	22	14	36
63–60	25	19	37
59–56	28	19	33
55–52	26	19	33
51–48	28		34
47–44	17		36
43–40	19		42
40–43	15		46
44–47	7		40
48–51	13		40
52–55	9	7	45
56–59	3	5	57
60–63	1	2	37
64–67	2	2	38
68–71	2	2	36
72–75	1	2	36
76–79	0		35

The record (fig. 2) obtained from this subject is very uniform throughout: and conforms very closely to what one might consider as being an ideal record. The measurements of this record are likewise uniform in that during decreasing temperature a gradual increase in the height of the knee-jerk was obtained until a higher level was established, which in this instance was approximately at a temperature of 64°F. (fig. 2). Likewise an increasing temperature reëstablished the normal height of the knee-jerk, which in this instance occurred at approximately 60°F.

Subject F is a Sophomore in college, weighs 145 pounds, is 20 years

old and is in good physical condition. He is slightly above the average in scholastic standing and seems to have about the average temperament. The record obtained from him (fig. 3) shows the least response to cold of any. For this reason it is of interest to note the uniformity in the general increase and decrease in the height of the knee-jerk during the falling and during the rising temperature. This subject also showed the usual increase

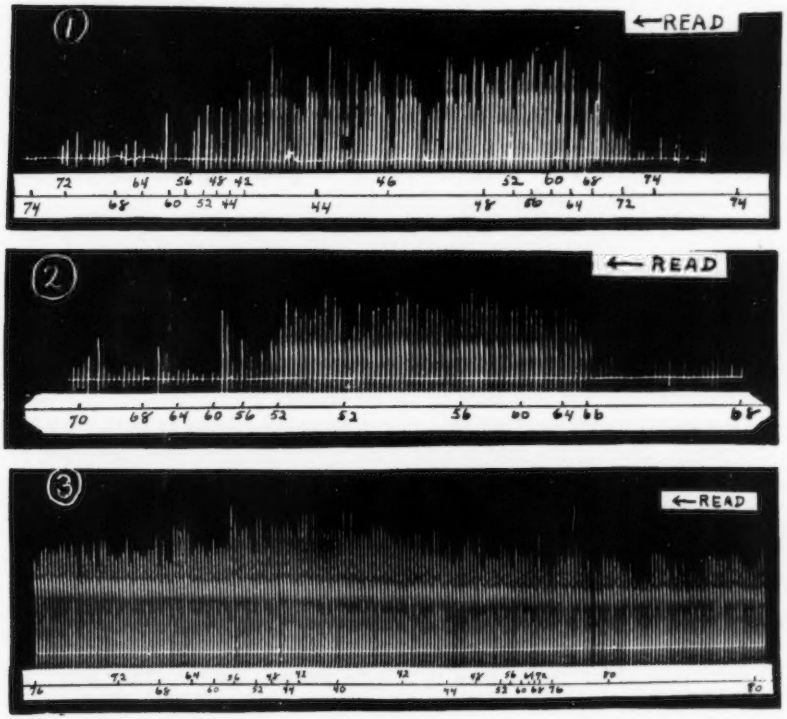

Fig. 1. Record of subject R showing the height of the knee-jerk for every four degrees Fahrenheit change in temperature.

Fig. 2. Record of subject H under same conditions.

Fig. 3. Record of subject F under same conditions.

in muscle tonus during the first few degrees fall in temperature as represented by the increase in the height of the knee-jerk. A corresponding decrease in muscle tonus was especially evident during the first few degrees rise in temperature which was, in this instance, 44° to 47°F. (table 3).

Discussion. It is known (Lombard (1887), Bodwich and Warren (1890), Tuttle (1924 b) and others that the knee-jerk may be reën-forced by anything that attracts the attention. These influences

were excluded as far as possible by doing the experiments at night and working in silence. After the record was taken the subjects were questioned concerning the experiment. They all expressed surprise at the records obtained during the cold period, thus showing they had not voluntarily increased the height of the knee-jerk.

It has been shown by Bodwich and Warren (1890), that a draft of air blown upon a subject will reënforce the knee-jerk. For this reason the average height of the knee-jerk may have been influenced by the air blown from the electric fan during the cold period (table 1). In order to make the draft of air from the fan a constant factor the fans were kept going throughout the experiment (tables 2 and 3). The data presented in this paper indicate that the increase in tonus caused by the electric fans is due to a change in temperature brought about by evaporation, radiation and the distribution of the temperature change.

It will be seen, table 1, subjects E and B, December 29, that the height of the normal knee-jerk is equal to that of the cold period. These exceptions to the other data are probably due to the fact that in this instance both subjects had been walking in the cold and had been in the laboratory only about five minutes before the experiment was begun. Thus it seems that the normal knee-jerk in these instances was increased by the stimulation from the cold walk to the laboratory. .

It is generally known that cold increases muscle tonus as in the case of shivering. During the cold period some of the subjects were observed to shiver and in these instances the height of the knee-jerk was at its maximum. This is in accordance with the idea that the height of the knee-jerk is an index to the phenomenon of muscle tonus.

CONCLUSION

The data presented in this paper indicate that a decrease in temperature is accompanied by an increase in muscle tonus. On the other hand an increase in temperature is accompanied by a decrease in muscle tonus.

The most abrupt change in tonus takes place during the first four to eight degrees change in temperature.

A draft of air reinforces the change in tonus brought about by a change in temperature which seems to be due to: a, evaporation; b, radiation; and c, distribution of temperature.

The writer wishes to express his indebtedness to Dr. W. W. Tuttle for use of his apparatus and for suggestions in carrying out this experiment.

BIBLIOGRAPHY

BOWDITCH, H. P. AND J. W. WARREN. 1890. Journ. Physiol., xi, 25.
LOMBARD, W. P. 1887. Amer. Journ. Psychol., i, 5.
RUGG, H. O. 1917. Statistical methods applied to education. 110. Houghton, Mifflin Co.
TUTTLE, W. W. 1924a. This Journal, lxviii, 338. 1924b. Journ. Exper. Psychol., vii, 401.

CONTRIBUTIONS TO THE PHYSIOLOGY OF GASTRIC SECRETION

VII. The Elimination of Dyes by the Gastric Mucosa

A. B. DAWSON and A. C. IVY

From the Department of Anatomy, Loyola University School of Medicine, and the Hull Physiological Laboratory, the University of Chicago

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During the past twenty years attention has been directed, somewhat sporadically, to the question of the elimination of dyes by the gastric mucosa. It is believed by some that the degree, manner and rate of dye elimination may be used as a valuable supplementary test of secretory activity, as an aid in the diagnosis of carcinoma and ulcer of the stomach, and also as a method of analyzing the gastric secretory process.

Schlaepfer (1908) utilized neutral red as an indicator of stomach activity by mixing the dye with the food and observing the time and rate of the appearance of the dye in the urine. His claim to originality in this method is disputed by Fuld (1908) who referred to Muenier's (1904) "Eisenchlorideprobefrühstück" as a similar method of estimating gastric activity. Fuld, in his experimental work, used Pavlov pouch dogs and placed the dyes in the "large" stomach. He found that neutral red appeared in the pouch within twenty-four hours after it was placed in the stomach. Pyronin in his hands proved very toxic, killing the dogs in a short time. Harvey and Bensley (1912) employed vital dyes and from their observations it could be concluded that neutral red, naphthol blue and nile blue sulphate were eliminated by the gastric mucosa. They, however, were not primarily interested in dye elimination. Polychrome B was used by Collip (1920). He found that it was toxic in large doses and that it stained the gastric mucosa. He, however, does not definitely state that the dye appeared in the secretion.

The more recent interest in this problem was apparently initiated by Saxl and Scherf (1922) who found that methylene blue was eliminated and that indigo carmine did not appear in the secretion. They introduced the dyes subcutaneously, intravenously and intraperitoneally. Rosenthal and von Falkenhausen (1922) denied that methylene blue, injected subcutaneously, would appear in the gastric secretion. Held (1923) was able to recover traces of methylene blue in the pouch if a sufficiently large quantity was introduced by subcutaneous injection. Finkelstein (1923),

working with Pawlow-pouch dogs, injected eight dyes subcutaneously and found that only one, neutral red, was eliminated in the secretion of the pouch. The neutral red appeared twenty-five minutes after the injection of 50 mgm. of the dye. The other dyes, indigo carmine, congo red, rhodamin, eosin, phenolsulphonephthalein, acridin red and phenoltetrachlorphthalein were not eliminated. In a second paper Saxl and Scherf (1923) reported that neutral red and methylene blue were eliminated and that indigo carmine, congo red, flourescin and pyrrol blue were not eliminated. They expressed the opinion that only dyes soluble in both lipoids and water are eliminated.

Fricker (1923) has injected 4 cc. of a 4 per cent solution of indigo carmine in normal saline into the gluteal muscles in man. He found that no elimination occurred in the stomachs of 5 healthy persons, of 4 cases of chronic gastritis and of 1 case of simple achylia, but in 5 cases of carcinoma of the stomach (verified at autopsy) and in two cases of peptic ulcer, he obtained a distinct blue color in the gastric secretion. He concluded that the elimination of a dye which does not normally appear in the gastric secretion is evidence of carcinoma or ulcer of the stomach.

Glaessner and Wittgenstein (1923) were able to confirm the results of other workers for neutral red. Later Simici and Dumitriu (1924) experimented with eight dyes, using in their tests thirty-eight healthy individuals. They administered 50 mgm. of the dye intramuscularly. Neutral red, methylene blue and rhodamin were eliminated, making their appearance in the stomach in from 10 to 45 minutes after their administration. Indigo carmine, congo red, eosin, phenolsulphonephthalein and methyl violet did not appear in the stomach. Carnot and Gaehlinger (1924) have injected neutral red and methylene blue intramuscularly in patients. They found that the elimination of the dye was accelerated in hyperchlorhydria, retarded in hypochlorhydria, and absent in achlorhydria. Hirabayashi (1924) studied the elimination of neutral red in dogs, following ingestion of an Ewald meal and found that the elimination of the dye occurred 2 to 5 times more rapidly when the meal was followed by the injection of either pilocarpin, histamine or spinach secretion, which are gastric secretory excitants.

For over two years we have been conducting a series of experiments on dogs with an isolated pouch of the stomach in an attempt to determine the site of origin of the hydrochloric acid. In this work we have experimented with a large number of dyes which were known to be of value either as vital stains or as indicators of hydrogen ion concentration. Very few of these dyes proved favorable for the study of acid formation and the observations recorded in this report may accordingly be regarded as the by-product of the original and more fundamental problem. However, in performing these experiments we have been able 1, to confirm the findings

of previous observers; 2, to extend considerably the list of dyes which are eliminated by the gastric mucosa with the hope that our results might throw some light on the mechanism of dye elimination; and 3, to make observations on the toxicity of dyes that are used to such a great extent by histologists. Further, we have attempted to test systematically all available representatives of the different groups of dyes, Mann's (1902) classification being followed almost entirely.

METHODS. In our experiments we have used dogs with an isolated fundic pouch (Pavlov pouch) and dogs with an isolated pyloric pouch (Ivy, 1921), the latter animals being used to ascertain if certain dyes were eliminated by the mucous cells.

Obviously the pouch preparation has several distinct advantages over other methods. First of all the effect of the injection of the dye on the rate of gastric secretion can be followed at all times. Secondly, there can be no possible contamination of the gastric secretion by dye eliminated through other organs, i.e., by the swallowing of dye eliminated in the mouth or the regurgitation of dye eliminated through the liver, intestine or pancreas. Also, the presence of ulcers or pathology of the gastric mucosa which might jeopardize a normal response can be readily detected by inspection of the pouch.

The Pavlov pouch was activated by a meal of ground meat and at times when the rate of secretion was low 1 mgm. of histamine was injected subcutaneously. The pyloric pouch was activated when necessary by the continuous application of gastric juice.

We noted the elimination of some of the dyes in the urine and feces, but on this point our observations are not complete.

All the dyes were injected intravenously at a slow rate. They were dissolved, except in one instance, in physiological salt solution, a saturated solution of the dye being used in most instances. The amount injected was recorded and symptoms of toxicity noted. The gastric juice was collected and titrated. The time of the appearance of the dye in the secretion was carefully ascertained. The dose of the various dyes was variable, because of differences in solubility and toxicity.

The dyes used in our work were obtained from the following sources: P. Gruebler & Co. (ante bellum): dahlia, bismarck brown, congo red, malachite green, neutral red, pyronin, safranin, thionin, toluidin blue, tropaeolin 00. P. Gruebler & Co. (post bellum): indulin (water soluble).

Coleman and Bell: alizarin blue S, alizarin red S, aurin, azolitmin, basic fuchsin, eosin Y, indigo carmine, janus green, nile blue sulphate, and trypan blue.

National Aniline and Chemical Co.: acid fuchsin, methyl green (vital), naphthol blue, nile blue chloride, pyrrol blue and rhodamin B.

H. A. Metz and Co.: quinoline blue (cyanin).

Merck and Co.: methylene blue (medicinal).

RESULTS. We will classify our results on the elimination of thirty-three dyes by the gastric mucosa according to Mann's (1902) chemical classification of the dyes. Only brief protocols will be given.

SERIES I, with Pavlov pouch dogs.

I. The Azo Dyes:

1. *Methyl Orange* (*Tropaeolin D, Orange III*), a mono-amido compound of the monazo group. Injected 50 cc. of a saturated solution. This dye is not freely soluble in normal saline. No elimination. No depression of secretion.

2. *Tropaeolin OO*, a mono-amido compound of the monazo group. 40 cc. of a saturated solution. No elimination, no inhibition of secretion, no evidence of toxicity.

3. *Bismarck Brown*, a diamido compound of the monazo group. Injected 25 cc. of a saturated solution. Visible in secretion in 10 minutes. Secretion a definite brown color in 20 minutes. Traces in secretion 24 hours later.

4. *Janus Green*, a monazo dye combined with the azonium chromophore of safranin to form a compound safranin-azo dye. Injected 20 cc. of a saturated solution. Very toxic; salivation, convulsions, analgesia, paralysis, relaxation of sphincters, pulmonary edema, death within 10 minutes after injection.

5. *Congo Red*, a benzidin-tetrazo compound. Injected 30 cc. of a 1 per cent solution. No elimination. No inhibition of gastric secretion. Not toxic.

6. *Pyrrol Blue*, a benzidin dye. Injected 25 to 30 cc. of a saturated solution. No elimination in the pouch. Slight depression of secretion. Dog vomited 2 hours later. Vomitus stained deep blue. Feces blue, urine blue. Appearance in vomitus probably due to regurgitation.

7. *Trypan Blue*, a benzidin dye. Injected 30 cc. of a saturated solution. Secretion normal. No elimination.

8. *Chrysoidin Y* a hydrochloride of diamido-azo-benzene. Injected 50 cc. of a saturated solution. Dye eliminated in 5 minutes.

II. *The Oxyquinones*:

1. *Alizarin Red S.* Injected 45 cc. of a saturated solution. No depression of secretion, no elimination through the pouch. Urine red.

2. *Alizarin Blue S.* Injected 40 cc. of a saturated solution. Dog vomited 5 minutes later. Secretion depressed. No elimination.

III. *The Quinone-imide Dyes*:

A. *The Thiazins*:

1. *Thionin.* Injected 25 cc. of a saturated solution. Appeared in secretion in 5 minutes. No depression of secretion.

2. *Toluidin Blue.* Injected 15 cc. of a saturated solution. Dog vomited within 3 minutes. Dye present in vomitus. Dye also appeared in the secretion of the pouch, the flow from which was completely inhibited in 5 minutes.

3. *Methylene Blue.* Injected 10 cc. of a saturated solution. Dye appeared in the secretion in 10 minutes. Secretion normal or slightly augmented.

B. *The Oxazins*:

1. *Naphthol Blue.* Injected 40 cc. of a saturated solution. Secretion colored red in 10 minutes. No depression of secretion. Urine colored fourteen hours later.

2. *Nile Blue Chloride.* Injected 25 cc. of a saturated solution. Dye not readily soluble in salt solution. Traces appeared in the pouch in 20 minutes. No evidence of toxicity.

3. *Nile Blue Sulphate.* Injected 40 cc. of a saturated solution in distilled water.

Dye eliminated in 40 minutes. No depression of secretion. The sulphate is practically insoluble in normal saline.

4. *Cyanamin Bichloride.*[1] Injected 50 cc. of a saturated solution. Dye sparingly soluble in salt solution. Traces appeared in the pouch in 25 minutes. No evidence of toxicity.

C. *The Azins*:

(a) Amido-azins:

1. *Neutral Red*, a diamido azin. Injected 50 cc. of a saturated solution. Appeared in secretion in from 3 to 5 minutes. No effect on rate of secretion. Not toxic. Appeared in urine.

(b) The Safranins:

1. *Safranin.* Injected 50 cc. of a saturated solution. Skin and oral mucosa dyed a bright red. Slightly toxic, causing muscular twitchings, salivation and sometimes vomiting. Secretion not inhibited. Appeared in urine. No elimination in pouch.

D. *The Indulins*:

1. *Indulin* (water soluble). Injected 500 mgm. in normal saline. Dog vomited several times. No elimination. No inhibition of secretion. Urine blue-green color. Feces normal color.

IV. *Phenyl-methane Derivatives.*

A. *Diphenyl-Methanes*:

1. Pyronin, a diphenyl-methane. Injected 15 cc. of a saturated solution. Acceleration of respiration, rigidity of neck, salivation and excitement. No elimination of the dye. Did not use a larger dose because of toxicity.

B. *Triphenyl-Methanes*:

(a) Rosanilin Derivatives.

1. *Malachite Green.* Injected 20 cc. of a saturated solution. Salivation and muscular twitching immediately after injection. Secretion continued. Minute trace in gastric juice in 30 minutes. In 60 minutes secretion has a slight but well defined green tint.

2. *Methyl Green.* Injected 5 cc. of a saturated solution. Extremely toxic, causing vomiting, urination, defecation, analgesia and complete prostration. Recovery complete in 45 minutes. Urine green.

3. *Basic Fuchsin.* Injected 40 cc. of a saturated solution. No elimination. Not toxic.

4. *Acid Fuchsin.* Injected 500 mgm. of normal saline. Oral mucosa, sclera and skin dyed red. Not toxic. Secretion tinted in 20 minutes. In 40 minutes the secretion was slightly but definitely colored red. After 80 minutes dye was still being eliminated. Appeared in urine.

5. *Hoffmann's Violet (Dahlia).* Injected 50 cc. of a saturated solution in normal saline. Not toxic. Secretion not depressed. No elimination.

(b) *Rosolic Acid Derivatives*:

1. *Aurin.* Injected 40 cc. of a saturated solution. The dye is practically insoluble in distilled water but is slightly soluble in normal saline. No elimination in pouch. No depression of secretory activity.

(c) *Phthaleins*:

1. *Eosin.* Injected 500 mgm. Not eliminated. Not toxic.

2. *Mercurochrome.* Injected 75 mgm. Not eliminated. Not toxic in this dose when given to a 15 kilo dog.

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[1] We are indebted to Dr. W. C. Austin of the Department of Chemistry, Loyola University School of Medicine, for the preparation of this dye.

3. *Rhodamin B.* Injected 40 cc. of a saturated solution. Gastric secretion a distinct red color in 30 minutes. Not toxic.

V. *Quinoline Compounds.*

1. *Cyanin* (Quinoline Blue). Injected 200 mgm. in 50 cc. normal saline. Skin and oral mucosa stained blue. No elimination in pouch. Not toxic. Feces and urine blue.

VI. *Natural Dyes.*

1. *Indigocarmine.* Injected 25 cc. of a saturated solution. Secretion inhibited. Slight secretory response to histamine. No elimination in pouch.

2. *Azolitmin.* Injected 30 cc. of a saturated solution. Dog vomited and defecated within 5 minutes. Secretion not inhibited. No elimination in pouch. Slightly toxic. Appeared in urine.

SERIES II, with pyloric pouch dogs.

1. *Neutral Red.* Injected 50 cc. of a saturated solution. Pouch not active. No elimination. Injected the same amount and activated the pouch by continuous application of gastric juice. Dye appeared in the pyloric pouch in 15 minutes.

2. *Nile Blue Sulphate.* Injected 40 cc. of a saturated solution in distilled water. Pouch activated as before. No elimination in 3 hours.

3. *Malachite Green.* Injected 50 cc. of a saturated solution. Pouch was secreting actively. No elimination in 3 hours.

4. *Acid Fuchsin.* Injected 50 cc. of a saturated solution. Dog in which vomiting is easily induced, vomited 5 times within the first 20 minutes. Pouch was active at the time of injection and remained so. No elimination in 3 hours.

SUMMARY OF RESULTS. The results on the elimination of the dyes are summarized in table 1.

Of the thirty-three dyes studied thirteen were eliminated in the gastric juice. Six of the thirteen required a latent period of thirty minutes or more before they became manifest in the secretion. Those dyes that appeared after a short latent period reached a much higher concentration in the gastric juice than those that appeared after a long latent period. The amount of dye eliminated is related to the rate of secretion, i.e., the more gastric juice secreted, the more dye eliminated.

All of the dyes appeared in the urine or feces or both, in those instances in which particular attention was directed to this matter.

Of the four dyes injected into dogs with a pyloric pouch only one, neutral red, was eliminated and then only when the mucosa of the pouch was activated with gastric juice.

Twelve of the dyes used gave evidence of toxicity. Janus green, methyl green and pyronin proved to be markedly toxic. Toluidin blue, alizarin blue S, safranin and malachite green were mildly toxic and pyrrol blue, indulin, indigo carmine, azo-litmin and acid fuchsin were only slightly toxic. To our surprise some of the mildly toxic dyes appeared in the gastric juice. We could not predict the effect of a dye from a study of its chemical constitution.

DISCUSSION. Our results confirm the observations of different investigators who found that neutral red, methylene blue and rhodamin are eliminated and that pyronin, indigo carmine and congo red are not elimi-

TABLE 1

Summary of results on the elimination of dyes by the gastric mucosa

Pavlov pouch dogs

DYES THAT ARE ELIMINATED	TIME	DYES THAT ARE NOT ELIMINATED
	minutes	
I. Azo Dyes.		I. Azo Dyes
Bismarck brown—hydrochloride	10	Methyl orange—sulphonated
Chrysoidin y—hydrochloride	5	Janus green—azo safranin—*very toxic*
		Congo red—sulphonated
		Pyrrol blue-base—*slightly toxic*
		Trypan blue
		Tropaeolin 00—sulphonated
II. Oxy-quinones		II. Oxy-quinones
		Alizarin red S—sulphonated
		Alizarin blue S—Na bisulphite *mildly toxic*
III. Quinone Imides		III. Quinone Imides
Thiazines:		
Thionin—hydrochloride	5	Safranins:
Toluidin blue—chloride—*mildly toxic*	3	Safranin—chloride—*mildly toxic*
Methylene blue—chloride	10	
Oxazines:		Indulins:
Naphthol blue—chloride	10	Indulin—water soluble-sulphonated—*slightly toxic*
Nile blue chloride—slight elimination	20	
Nile blue sulphate—slight elimination	40	
Cyanamine bichloride—slight elimination	25	
Azines:		
Neutral red—hydrochloride	3	
IV. Phenylmethanes		IV. Phenylmethanes
Di-phenylmethanes		Di-phenylmethanes
		Pyronin—chloride—*very toxic*
Tri-phenylmethanes		Tri-phenylmethanes
Rosanilin derivatives:		Rosanilin derivatives:
Malachite green—chloride—slight elimination—*mildly toxic*	30	Methyl green—chloride—*very toxic*
		Basic fuchsin—hydrochloride
Acid fuchsin—sulphonated—slight elimination—*slightly toxic*	40	Dahlia (Hoffmann's violet) -hydrochloride
		Rosalic acid derivatives:
		Aurin—very slightly soluble
Phthaleins:		Phthaleins:
Rhodamin B—chloride	30	Eosin-bromine
		Mercurochrome—bromine and mercury
V. Quinolines		V. Quinolines
		Cyanin-iodide
VI. Natural Dyes		VI. Natural Dyes
		Indigo-carmine—*slightly toxic*
		Azo-litmin—*slightly toxic*

nated by the gastric mucosa. We have not repeated the experiments of others who have used methyl violet, fluorescin, phenolsulphonephthalein and phenoltetrachlorphthalein and have found that they were not eliminated, but we have ample reason to believe that their observations are correct because we have found only one phthalein that is eliminated, namely rhodamin, and also because methyl violet, which is not a phthalein, usually occurs as an impurity in methyl green which was not eliminated in our experiments. We did not use acridin red, which is not eliminated by the gastric mucosa (Finkelstein, 1923), but did use a quinoline, cyanin, which also is not eliminated.

The amount of any dye which will be eliminated and the rate of elimination is probably dependent on many factors. It seems logical to assume that one of these factors is the rapidity with which the dye is lost from the blood stream through other exits such as the liver and kidney. For example, pyrrol blue, alizarin, safranin, indulin, phenolsulphonephthalein are readily eliminated by the kidney, but do not appear in the gastric secretion. Phenoltetrachlorphthalein and indigo carmine are readily eliminated by the liver, but not by the gastric mucosa. On the other hand, either the chemical or physical nature of the dye seems to be a factor as methylene blue, for example, is eliminated by all three of the organs here considered and neutral red by both stomach and kidney and possibly the liver. A study of the chemical structure of the dyes fails to throw any light on our problem. Solubility is a factor which must be considered as it determines the concentration of the dye in the blood stream. However, in several cases, the solubilities of the two dyes, e.g., malachite green and neutral red, were not unlike, but the length of time taken for the appearance of the color in the pouch was decidedly different. Cyanin was quite soluble and stained the animal deeply blue, but was not eliminated by the gastric mucosa.

There was a time in the course of our experiments that it appeared as if only dyes that were in the form of a water soluble chloride or hydrochloride were eliminated. But we found that nile blue sulphate, and acid fuchsin (sulphonated) were eliminated, whereas basic fuchsin (hydrochloride), safranin and dahlia were not eliminated. It is to be noted that safranin and methyl green (chlorides that were not eliminated) are toxic, and that nile blue sulphate was injected unphysiologically (in distilled water) and that acid fuchsin was also toxic, are facts that would possibly warrant the conclusion that the results on these dyes should be considered abnormal and hence do not cast doubt on our chloride hypothesis. Granting this possibility, we are still unable to explain why basic fuchsin and dahlia, both being water soluble hydrochlorides, are not eliminated. Hence, a chloride hypothesis is inadequate.

Saxl and Scherf (1923) state that only dyes which are soluble in both

lipoids and water are eliminated by the gastric mucosa. They apparently based their conclusion on the work of Overton (1900). Overton tested many of the best known vital dyes and found that dyes which stained living cells readily were soluble in lecithin, cholesterin, protagon and cerebrin, the so-called cell lipoids, but that those dyes which are not vital stains were insoluble in oils, fats and fatty acids. Many exceptions to the rule of the lipoid-solubility of dyes which pass into living cells have been found, the work of Ruhland (1912) containing many examples. He tested thirty basic and eighty-nine acid dyes.

Our results with Bismarck brown, thionin, toluidin blue, methylene blue, neutral red, malachite green and rhodamin B. would support Overton's hypothesis since these were found by him to be lipoid soluble and were eliminated by the stomach. But he also found that safranin, methyl green and dahlia were soluble in lipoids and in our experiments these were not eliminated by the stomach. Further, we found that acid fuchsin, a sulphonic acid dye, appeared in the gastric juice, but this is a dye which, according to Overton, is not lipoid soluble. It should also be noted that indulin and indigo carmine, both sulphonic acid dyes, which are insoluble in lipoids appeared in the urine, but did not appear in the pouch. We have not been able to obtain data on the solubilities of all the dyes tested in our experiments but the exceptions which have been pointed out are sufficient to show that the lipoid constituents of the animal cell membrane is not the only factor which determines the permeability of a cell.

Selective secretion is another obvious factor that must be considered. In an organ with such a diverse histological structure as the stomach it is difficult to be positive that all the dyes are being eliminated by the same cells. That is, some dyes may possibly be eliminated through the parietal cells, others through the chief cells or mucous cells. Further, the fundic mucosa may not be the only portion of gastric mucosa which participates in the elimination of the dyes. The pyloric mucosa may also be active. It has been assumed by several workers that the fact that dyes are not eliminated by a resting mucosa, but pass out through an active one, is sufficient evidence that only the fundic mucosa is involved in this process. Ivy and Oyama (1921) have shown, however, that the normal stimulus for the activation of the pyloric mucosa is gastric juice, especially its acid component. Accordingly in a resting stomach, i.e., one which is not actively secreting acid, the pyloric mucosa may also be inactive. The wide variation in the time required for different dyes to appear in the pouch might therefore be explained on the ground that some dyes are eliminated by one type of epithelial cell and others by a different type of cell.

Several dyes, of which neutral red is the best example, can be detected by microscopic examination to be passing out through the parietal cells as the secretion in the canaliculi is rendered conspicuous by the dye. Other

dyes which are liberated more slowly cannot be detected with certainty in any part of the mucosa. Both chief cells and mucous cells contain considerable amounts of stored prosecretion.

Our results on the elimination of dyes by the pyloric mucosa (experiments under series II) show that the mucous cells of the stomach play a minor rôle, if any, in the elimination of the dye. Neutral red, which appears within five minutes in the secretion of the fundic pouch, is only eliminated by the mucous cells after activation with gastric juice and even then at a very slow rate. Nile blue sulphate, malachite green and acid fuchsin, which are eliminated slowly and to only a slight extent by the fundic pouch, are not eliminated by the mucous cells of the pyloric antrum. It is apparent then that the slow elimination of some dyes is not due to the possibility of their being eliminated by mucous cells and that neutral red, which is most readily eliminated by the parietal cells, can under certain conditions be eliminated by the mucous cells of the stomach.

It appears, then, from the above facts that the parietal cells are the cells chiefly concerned in the elimination of dyes by the gastric mucosa and whether or not a dye is eliminated by these cells depends on some unknown as yet physical rather than chemical property of the dye, as there is no chemical property characteristic of the dyes that are eliminated.

Although the amount of neutral red and other dyes that are eliminated is a function of the rate of gastric secretion, we do not believe that any more accurate knowledge can be obtained by determining the amount of dye eliminated than can be obtained by the usual test meal. If regurgitation from the intestine is ruled out by testing gastric contents for bile, we believe that Fricker's test (1923)—the occurrence of indigo carmine in the gastric contents of patients with ulcer and carcinoma of the stomach—should prove of some practical value since this dye is not eliminated by a normal gastric mucosa. Nevertheless, it seems to us that the problem of dye elimination by the gastric glands has a greater scientific import than practical value.

CONCLUSIONS

1. The following thirteen dyes are eliminated by the gastric glands: neutral red, toluidin blue, thionin, methylene blue, chrysoidin y, naphthol blue, bismarck brown, nile blue chloride, cyanamin bichloride, rhodamin, malachite green, acid fuchsin, nile blue sulphate, the first seven being quite rapidly and readily eliminated. Twenty other dyes from various chemical groups were found not to be eliminated.

2. We have been unable to find any constant chemical or physical property that might explain why the above dyes are eliminated, but discuss our results from the standpoint of the mechanism concerned.

3. The parietal cells are the cells of the gastric mucosa most intimately concerned with dye elimination.

4. Twelve of the thirty-three dyes used gave evidence of being toxic. They are as follows: janus green, methyl green, pyronin, toluidin blue, alizarin blue S, safranin, malachite green, pyrrol blue, indulin, indigo carmine, azo-litmin and acid fuchsin, the first three being decidedly toxic.

BIBLIOGRAPHY

CARNOT ET GAEHLINGER. 1924. Paris Médical, November 8, 377.
COLLIP, J. B. 1920. Univ. Toronto Studies, Physiol. Series, no. 35.
FINKELSTEIN, R. 1923. Arch. f. Verdauungskr., xxx, 299.
FRICKER, E. 1923. Arch. f. Verdauungskr., xxxii, 183.
FULD, E. 1908. Muenchen Med. Wochenschr., lv, 2264.
GLAESSNER, K. AND H. WITTGENSTEIN. Wien. klin. Wochenschr., xxxvi, 791.
HARVEY, B. C. H. AND R. R. BENSLEY. 1912. Biol. Bull., xxiii, 225.
HELD, cited by FINKELSTEIN, 1923.
HIRABAYASHI, N. 1924. Arch. f. Verdauungskr., xxxiii, 71.
IVY, A. C. AND Y. OYAMA. 1921. This Journal, lvii, 51.
MANN, G. 1902. Physiological histology, Oxford.
MEUNIER. 1904. Cited by FULD, 1908.
OVERTON, E. 1900. Jahrb. f. wiss. Bot. xxxiv, 669.
ROSENTHAL UND VON FALKENHAUSEN. 1922. Wien. klin. Wochenschr., xxxv, 442.
RUHLAND, W. 1912. Jahrb. f. wiss. Bot. li, 376.
SAXL, P. AND D. SCHERF. 1922. Wien. klin. Wochenschr., xxxv, 129. 1923, Ibid., xxxvi, 671.
SCHLAEPFER. 1908. München. Med. Wochenschr., lv, 1865.
SIMICI, D. ET DUMITRIU. 1924. Arch. des Maladies de l'App. Digest, xiv, 310.

ADDENDUM

Since sending in this manuscript we have obtained and used two other dyes, namely, neutral violet and benzopurpurin 4B.

Neutral violet differs chemically from neutral red only in having a methyl group less. It is slightly less soluble in 0.9 per cent NaCl solution than neutral red. When 50 cc. of a saturated solution is injected neutral violet does not appear in the gastric secretion. On the injection of a supersaturated solution containing 300 mg. of the dye, no elimination occurs. This negative result is especially interesting in view of the slight chemical difference between neutral red, which is eliminated copiously by the gastric glands, and neutral violet.

Benzopurpurin 4B is closely related to congo red. Congo red is not eliminated by the gastric glands, neither is benzopurpurin 4B.

HEAT REGULATION AND WATER EXCHANGE

VII. Evidence that Cold Anhydremia is Due to Loss of Fluid from the Blood Stream[1]

H. G. BARBOUR and W. F. HAMILTON

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville

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Increased blood concentration has been clearly established as one of the constant protective responses of the body to environmental cold (1), (2). There is, however, no decisive evidence on record as to whether this cold anhydremia is due to the movement of blood cells or fluid. The former event entails two possibilities: cell accumulation in those parts of the blood stream from which the samples have been taken, or a general movement of cells into the whole blood.

An example of the arguments which have been advanced in favor of cell accumulation is seen in the contention of Bostrom (3) that the forms of anhydremia produced by cold, epinephrin and asphyxia are all due to local peripheral stasis of cells. On the other hand, Edmunds and Stone (4) argue that epinephrin anhydremia is due to hematopoiesis. It will, however, be shown later that the adrenals have little or no significant connection with cold anhydremia.

The hypothesis of uneven distribution of cells within the blood stream can be discarded if it can be proven that the blood as a whole concentrates.

In the following analysis the value of the ear as a source for samples of the actively-circulating blood under the conditions of our experiments will be established in particular by showing that the increased concentration in the ear samples is paralleled strikingly by that seen both in arterial blood and venous blood from the ankle.

Most of the samples showing cold anhydremia in the work reported here and elsewhere were taken from the dog's ear. Since in a dog immersed to the neck in a cold bath, the blood in passing from the heart to the ear vein is in no part of its path subjected either to stasis or to significant metabolic changes, it is obvious that blood emerging from any of the vessels of the ear, if flowing freely, will yield a good sample of the actively-circulating stream. If the systemic blood represented what was left after

[1] This investigation has been assisted by a grant from the Ella Sachs Plotz Foundation.

an increased proportion of cells had accumulated in a cooled area, it would, as shown in the conditions of Bostrom's anesthetized animals, exhibit dilution. In the evidence to be submitted further on it will again be made clear that in the unanesthetized dog exposed to a cold environment, the ear blood becomes concentrated. Evidence from anesthetized animals as to the normal blood concentration mechanism is valueless[2] (5).

It is now proposed to show from direct observations that the ear blood, under the conditions of our experiments, offers an exact sample of the actively-circulating blood as found in a good-sized artery—the femoral. This artery was tapped by an ordinary hypodermic needle inserted into it through the uninjured skin.

Under normal conditions the following results were obtained:

EXPERIMENT NUMBER	TIME	SPECIFIC GRAVITY*		SOLIDS	
		Artery	Ear	Artery	Ear
1	5:03½	1.0516			
	5:06	1.0509			
	5:10		1.0513		
	5:11		1.0509		
2	4:07½		1.0486		
	4:08				18.7
	4:09		1.0481		
	4:10	1.0481		18.4	
	4:10½	1.0481			
	4:11	1.0481			

* Falling drop method (2).

This dog was next immersed in a cold bath (22.5°C.) from 4:15 to 4:35 o'clock. Immediately after removal, while the animal was still wet and shivering, the following results were obtained:

EXPERIMENT NUMBER	TIME	SPECIFIC GRAVITY		SOLIDS	
		Artery	Ear	Artery	Ear
2	4:37	1.0499		19.1	
(cont.)	4:37	1.0496		19.1	
	4:40		1.0501		
	4:41½		1.0502		19.2

[2] The use of unanesthetized animals in the type of experiments cited in this and the following paper is entirely justifiable on humane grounds for none of the procedures (application of ice, cold baths and withdrawal of blood samples) produce any greater discomfort than the ordinary process of anesthetization.

Another dog was placed on an ice pack so that arterial blood could be drawn while the coldest stimulus was still being applied. This resulted as follows:

EXPERIMENT NUMBER	TIME	SPECIFIC GRAVITY		SOLIDS	
		Artery	Ear	Artery	Ear
3	2:46		1.0463		
Before ice application	2:51		1.0459		
	2:52		1.0454		
	2:54				17.5
During ice application	3:44		1.0486		
	3:45		1.0494		
	3:52		1.0489		
	3:59		1.0499		
	4:01		1.0505		18.18
	4:02		1.0496	18.28	
	4:03	1.0481		18.14	
		1.0497			

The above observations show clearly not only that the ear blood and arterial blood both become concentrated as a result of the cold exposure but also that simultaneous samples of the two are practically identical as to water content either before or after the exposure.

The results of local stasis with accumulation of cells in some particular region might conceivably in arterial blood be disguised by the mixture of blood from the rest of the body. In order therefore to show that such local accumulation of cells does not occur in connection with exposure to cold, the blood emerging from the cooled capillary bed should be directly examined. This has been done in five experiments. Below are detailed the percentage changes found in blood solids and hemoglobin from both ankle vein and ear samples resulting from cold baths at about 20°C.:

EXPERIMENT NUMBER	BATH DURATION	SOLIDS		HEMOGLOBIN	
		Ankle vein	Ear	Ankle vein	Ear
	minutes	*per cent*	*per cent*	*per cent*	*per cent*
4	30	+9	+7	+15	+8
5	30	+10	+10		
6	30	+15	+10	+29	+20
7	15	+10.9	+14	+8.7	+14
8	60	+2	+5.5	+8.5	+14
Average		+9.38	+9.3	+15.3	+14

If the cells had accumulated in the cooled area, dilution instead of concentration would constantly be present in the venous blood from that area. Not only was this condition never seen but the average effect upon the ankle blood consisted of an increase in concentration as great as the average concentration seen in the ear. This is supported by blood solids determinations in all cases as well as by hemoglobin readings in four of the five It would be superfluous in the face of the evidence cited thus far to examine the blood of the heart.

Having shown therefore that uneven distribution of the blood cells does not in any way account for the observed facts of cold anhydremia and that we are here dealing with the actively-circulating blood as a whole, we may next ask whether this whole blood exhibits a loss of fluid or a gain of cells.

If cold anhydremia were due to a gain of cells by the whole blood, this would either have to be due to the manufacture of new cells or old ones would have to appear from some reservoir. Against the first hypothesis is the fact that the change is far too rapid (it can be detected within a minute), and is immediately reversible either by returning the animal to room temperature or by the application of heat. Among the many observations made on this point we may cite those recorded in paper VI (6) and also in connection with the hot-room experiment described below.

Concerning the possibility of emergence of blood cells from a reservoir, the only significant situation whence this could occur, according to present physiological conceptions, is the spleen. De Boer and Carroll (7) have shown that the splenic reservoir is under control of the central nervous system, the nerve supply presumably being sympathetic. It is therefore of significance that we have already shown (2), (6) that the normal anhydremic response to various degrees of cold is not diminished by cutting both splanchnic nerves.

Aside from the importance of these findings in excluding the adrenal mechanism they also eliminate the effect of the sympathetic innervation of the spleen. To exclude the entire nervous mechanism of the spleen, we have performed the following experiment: A dog in which the spleen had, for other purposes, been entirely denervated sixty days before, by the method described by de Boer and Carroll (7), was given a cold bath at 15°C. After a stay of twenty-eight minutes in the bath a sample of blood from the ear showed as marked concentration as would have been expected from a normal dog:

	BLOOD SOLIDS	BLOOD SPECIFIC GRAVITY	HEMOGLOBIN
Before bath	16.57	1.0445	51
After bath	17.67	1.0461	54.5

These experiments have excluded the nervous mechanism of, and the adrenal effect upon the spleen, hence it seems highly improbable that cold drives cells out of the splenic reservoir.

But cold anhydremia is associated with a definite loss of fluid from the blood. In support of this may be cited the following determinations of the solids and specific gravity of the whole blood and serum which were made upon a dog exposed for about an hour to a bath rising in temperature from 20° to 23°C. The solids determinations both before and after the bath are given in duplicate to indicate their small probable error:

	TIME	BLOOD			SERUM		
		Specific gravity	Solids	Change	Specific gravity	Solids	Change
				per cent			*per cent*
Before bath	10:22	1.0527	19.43		1.0226	8.04	
			19.40			8.04	
After bath	12:00	1.0550	20.39		1.0231	8.135	
			20.40	+5		8.14	+1

Thus the average change in whole blood solids was 5 per cent while the serum increased its solids by about 1 per cent. The fluid leaving the blood appears therefore to be of a concentration somewhat less than that of serum. It must of course be still less concentrated than the whole blood, its loss therefore working a greater change in the latter.

In the second of these experiments a dog was exposed for 30 minutes to a bath rising in temperature from 14°C. to over 16°C. The following extract from the protocol shows not only the results of this exposure but of an immediately subsequent 32 minute exposure to a hot environment (temperature 34° to 33°C., wet-kata 7.9 to 3.2 millicalories per square centimeter per second):

	TIME	BLOOD			SERUM		
		Specific gravity	Solids	Change	Specific gravity	Solids	Change
				per cent			*per cent*
Before bath	3:20	1.0484	18.28			7.7	
			18.22			7.81	
After bath	3:50	1.0509	19.14		1.0227	8.14	
			19.29	+5		8.11	+5
After hot room	5:18	1.0479	17.50			7.36	
			17.55	−4	1.0204	7.34	−5

In this last experiment the solids content of both serum and blood exhibited nearly equal changes, indicating that the concentration of the

fluid leaving the serum under the cold influence was much lower than that seen in the first case.

The evidence that fluid is shifted in and out of the blood stream in heat regulation is strengthened by the result that the serum diluted in the hot room part of the experiment. This is only a confirmation of the results of numerous experiments in man from this laboratory (8), in which it was shown that in the phase of blood dilution in response to warm environments, the solids, nitrogen and specific gravity of the serum all diminish. Having, therefore, proven that the blood loses fluid, our next paper will deal with its fate.

SUMMARY AND CONCLUSIONS

1. Ear, arterial and venous blood of unanesthetized dogs exposed to cold baths all exhibit concentration to an equal degree. Cold anhydremia is, therefore, not due to redistribution of cells.

2. The concentration is quick and reversible and is, therefore, not due to hematopoiesis.

3. The concentration response to cold has been found undiminished after the splanchnic nerves have been cut as well as after denervation of the spleen. It, therefore, seems unreasonable to ascribe it to emergence of blood cells from a place of storage.

4. Serum as well as whole blood concentrates in cold anhydremia. This establishes definitely the importance of fluid movement out of the blood.

BIBLIOGRAPHY

(1) Barbour: Physiol. Reviews, 1921, i, 295.
(2) Barbour and Hamilton: This Journal, 1924, lxix, 654.
(3) Bostrom: This Journal, 1921, lviii, 195.
(4) Edmunds, and Stone: Arch. int. de Pharm. et de Therap., 1924, xxviii, 391.
(5) Barbour and Bourne: This Journal, 1924, lxvii, 399.
(6) Hamilton, Barbour and Loomis: Journ. Physiol., 1924, lix, 306.
(7) de Boer and Carroll: Journ. Physiol., 1924, lix, 312.
(8) Barbour, Hamilton, Dawson and Neuwirth: Proc. Amer. Physiol. Soc., This Journal, 1925, lxxii, 233.

HEAT REGULATION AND WATER EXCHANGE

VIII. The Fate of the Fluid Leaving the Blood in Cold Anhydremia[1]

W. F. HAMILTON and H. G. BARBOUR

From the Department of Physiology and Pharmacology, School of Medicine, University of Louisville

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The evidence that cold anhydremia is not merely relative but due to actual loss of fluid from the blood was presented in the paper immediately preceding this (1). Results will now be reported which indicate the destination of this fluid.

In considering the reservoirs which might take up fluid lost in the regulation against cold and from which it could be returned in hot environmental conditions, one can at once exclude the kidney where only a very limited amount of fluid could be stored and returned to the circulation. That the brain tissue takes some part was indicated in the first paper of this series (2). The cerebro-spinal reservoir for fluid must, however, be limited to the difference in volume between dilated and constricted vessels supplying the central nervous system.

The splanchnic nerve region contains several possible reservoirs. Since the concentration phenomenon is in some respects suggestive of sympathetic stimulation, it was thought that cutting the splanchnic nerve might throw some light upon the location of these reservoirs. We have, however, shown in the previous paper that splanchnotomized dogs exhibit as much cold anhydremia as normal dogs. This would seem to exclude the adrenal-liver mechanism of Lamson (3), and in all probability, both the walls and lumen of the intestine.

We therefore approached the question of the skin, subcutaneous tissue and muscles as reservoirs, feeling that they were very strongly indicated by exclusion.

It is of course impossible to obtain samples of living tissues of unanesthetized animals during experimental procedures. Evidence was therefore sought by producing unilateral exposure of animals to a cold stimulus in the hope of establishing a difference between the water content of the tissues of the cooled and uncooled sides. After taking control samples of

[1] This investigation has been assisted by a grant from the Ella Sachs Plotz Foundation.

the blood from normal dogs, they were made to lie upon a block of ice from which they were separated only by a water-tight sheet. After exposure of this sort for a period varying in the different experiments from

TABLE 1

Changes in water content of blood, skin and muscle in unilaterally cooled dogs

EXPERIMENT NUMBER	TIME		BLOOD		TOTAL SOLIDS OF TISSUES		
			Specific gravity	Total solids	Cooled side	Uncooled side	Difference
				per cent	*per cent*	*per cent*	*per cent*
1	3:45		1.0469	17.55			
	3:46	Right side of dog applied to ice pack					
	3:56		1.0499	18.63			
	4:02		1.0506	19.25			
	4:05	Killed with illuminating gas					
		* * * *					
		Shoulder skin			25.98	29.38	11.6
		Thorax skin			30.3	31.02	2.3
		Thigh skin			26.2	26.98	2.9
		Thigh muscle			20.5	20.9	1.9
2	2:54		1.0458	17.5			
	3:35	Right side applied to ice pack					
	4:01		1.0502	18.18			
	4:27		1.0484	17.89			
	4:29	Killed with illuminating gas					
		* * * *					
		Shoulder skin			27.5	29.78	7.7
		Shoulder muscle			20.8	22.15	6.1
		Thigh skin			27.85	27.0	
		Thigh muscle			20.47	21.28	3.8
3	2:49		1.0410	14.76			
	2:59	Right side applied to ice pack					
	3:06		1.0438	15.15			
	3:15		1.0443	15.55			
	3:17	Killed with injection of cyanide					
		* * * *					
		Shoulder skin			23.91	24.8	3.6
		Shoulder muscle			19.76	20.8	5.0
		Thigh skin			24.5	25.0	2.0
		Thigh muscle			20.2	20.5	1.5

20 to 50 minutes, blood was again withdrawn and the animal immediately killed. In order to eliminate the manner of killing as a causative factor, this was varied; it was done in two cases with illuminating gas and in a

third by intrapleural injection of potassium cyanid into or near the heart. Samples of skin (including subcutaneous tissues) and muscle were then immediately taken from several symetrically-located points on the two sides, as shown in the table. The skin and muscle samples were weighed at once and then dried at a temperature just over 100°C. to a constant weight. In eleven out of the twelve symmetrical pairs of tissue fragments the water content of the cooled side was definitely greater than that of the uncooled side, the changes varying from 1.4 to 11.6 per cent.

The usual increased concentration in the ear blood as indicated both by specific gravity and total solids was seen definitely in each instance and in one case this was controlled by taking parallel samples from the femoral artery. Most rigorous precautions were taken to prevent the muscles and skin thus treated from becoming contaminated with water from any outside source. Since the blood concentrates and the tissues of the cooled side are found to contain more moisture than those of the uncooled, the fate of the fluid can thus be adequately accounted for.

This accumulation of water in and under the skin in the regulation against cold may be of considerable value. The significance of loss of fluid from the actively-circulating blood has been repeatedly emphasized (1), (4) as a means of facilitating the reduction of the peripheral blood flow to save heat. This fluid which leaves the blood serves the additional function of increasing the thickness of the insulating tissues. Contained undoubtedly in small cutaneous and subcutaneous vesicles, it can not carry heat by convection and is hence as good an emergency insulator as could be found within the body. The "suit of clothes" (5) which protects those regions in which blood now actively circulates is thus further padded by water.

CONCLUSION

The fluid which leaves the blood in the regulation of the body against a cold environment is diverted chiefly to the cooled skin, subcutaneous tissues and muscles. In these situations it helps insulate the deeper-lying tissues.

BIBLIOGRAPHY

(1) Barbour and Hamilton: This Journal, 1925, lxxiii, 315.
(2) Barbour: This Journal, 1924, lxvii, 366.
(3) Lamson and Roca: Journ. Pharm. Exper. Therap., 1921, xvii, 481.
(4) Barbour and Tolstoi: This Journal, 1924, lxvii, 378.
(5) DuBois, E. F.: Metabolism in Health and Disease, Lea & Febiger, Philadelphia, 1924, p. 328.

THE INFLUENCE OF RELAXATION UPON THE KNEE JERK

EDMUND JACOBSON AND A. J. CARLSON

From the Hull Physiological Laboratory of the University of Chicago

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Various clinical methods employed especially in the therapy of functional neurology are essentially empirical. Obviously, such methods become more reliable and useful, if founded upon or corroborated by accurate laboratory experiments. A method to produce an extreme degree of neuro-muscular relaxation has recently been described (Jacobson, 1924). This method has been found effective in the therapy of certain nervous conditions (Jacobson, 1920–21). It has been shown that by this method both the striated and the smooth muscle of the esophagus can be relaxed (Jacobson, 1925).

So far as we know, the effect of extreme relaxation on the knee-jerk has not been studied. In 1875 Westphal and Erb described the use of the knee-jerk in diagnosis. In 1886, Mitchell and Lewis concluded that "the responsive jerk brought about by striking a stretched tendon is the most refined measure we possess of deciding as to the tone of muscle." Since that time the knee-jerk has been widely used as a test of functional as well as of organic condition of the nervous system. It is generally accepted that any reflex that can be studied with quantitative accuracy can be used as an index of functional conditions of the central nervous system. Accordingly it should be possible to measure progressive relaxation by its influence on the knee-jerk.

Lombard (1887) noted that the knee-jerk usually decreased during quiet or, as he termed it, cerebral inactivity and sleep, on the average about one-half. Lee and Kleitman (1923) observed that no knee-jerk was obtained in sleep, and Tuttle (1924) found that the knee-jerk was absent in deep sleep, while during light sleep low kicks were registered. This confirmed Bowditch and Warren (1890) and Piéron (1913).

It is well known that during "relaxation" as ordinarily produced in clinical examinations the knee-jerk is not diminished. In fact, it is usually increased. Simply telling the untrained individual to relax usually increases the knee-jerk, and this is readily explained because the individual usually holds his limbs tense before being told to relax, thus mechanically decreasing the jerk, as can be verified upon palpation of the flexor muscles.

When told to relax, the jerk becomes more brisk in character because the contraction of the quadriceps is unimpeded by their antagonists. Sometimes it is necessary to talk to the patient or to have him perform some simple act such as counting or looking out of the window in order to distract his attention from the limb so that he does not hold it too tensely. When patients with exaggerated knee-jerk are told to relax in this manner, the kick usually becomes very marked.

Procedure: The apparatus used has been previously described (Lee and Kleitman, 1923) but was somewhat modified. The spring-hammer was changed so that its excursion was increased to about 5 cm., and it was set off mechanically and noiselessly by pulling a string. The subject lay in a semi-reclining chair or on a canvas cot with the right recording leg at an angle of about 70°. The left leg was supported so as to make the general position most comfortable. Seven subjects were used. Two were the writers' and five were former patients who had received prolonged training at progressive relaxation.

Procedure was as follows: Control tests were taken with the subject resting quietly and relaxed in the ordinary sense. The subject generally chatted or did mental arithmetic in order to avoid extreme relaxation. No shifting or change of position of the trunk or limbs took place from the beginning of the period of experiment to the end, for this would interfere with the accuracy of results. After the control records were obtained, the subject was instructed to relax extremely, including the muscles of the trunk, limbs, neck, as well as the small muscles of the head such as those of the eyes and speech. Generally a pause of about 15 minutes was permitted for the subject to get extremely relaxed, but sometimes about 5 minutes sufficed. On the other hand disturbing noises from the outside sometimes led us to lengthen the period. On some occasions the subject appeared more tense than usual at the outset and a relatively longer time was given him to become relaxed. We assumed that the subject was relaxed when he appeared generally flaccid and in complete repose, while the eye-balls were motionless and the closed lids did not flutter or wink. In the completely relaxed state, the muscles of the tested limb were entirely flaccid to palpation and unresisting to a slight push from the operator, which would flex it or extend it easily, because of the limp state. The stroke of the hammer came noiselessly and unexpectedly at intervals of irregular length. After such a stroke during the relaxed period it was necessary each time to allow several minutes for the subject to relax again. Sometimes apparent restlessness made it necessary to increase the length of this interval. Needless to say the subject did not as a rule interrupt the period of relaxation by speaking, and the operator tried to act quite noiselessly. Following the period of relaxation, a control series was again

Fig. 1. (F. S.). Showing decrease of the knee-jerk during partial relaxation.

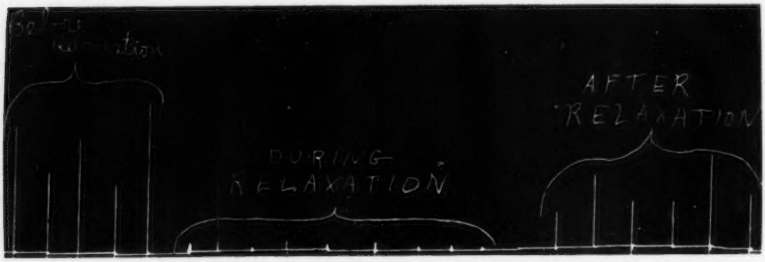

Fig. 2. (E. J.). Showing nearly complete absence of the knee-jerk in good relaxation.

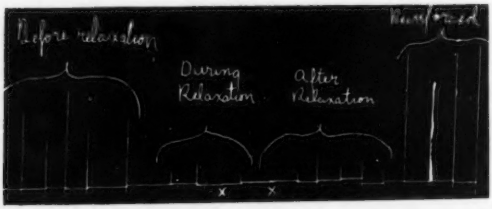

Fig. 3. (A. J. C.). Showing decrease of the knee-jerk to complete absence (x, x) by relaxation. At x, x, the subject was on the border of sleep, but not actually at sleep.

Fig. 4. (S. E.). Showing nearly complete absence of the knee-jerk in good relaxation.

taken. Here it was generally necessary to enliven the subject with conversation, for the relaxation tended to hold over.

Results: The records show a decrease of the jerk parallel to the degree of relaxation (figs. 1 to 4). In three of the subjects when relaxed to the greatest possible extent, the knee-jerk was usually absent. Our observation that the jerk, at least in some subjects, is absent in extreme relaxation and present but of diminished extent in relaxation not quite so extreme evidently parallels Tuttle's observations that the jerk is absent in deep sleep, but is present though diminished in light sleep. It should be emphasized here that our subjects even when extremely relaxed were not actually asleep, although in some cases they may be said to have been on the very border of sleep. Our results indicate that *by voluntary relaxation while still awake one may attain a degree of neuro-muscular tonus lower than that of light sleep.*

During advanced relaxation there was at least in some of the subjects a diminution of the respiratory and heart rate.

Study of individual records discloses various points of interest. A persistent noise, like a crackling steam-radiator in the room, may be relatively unnoticed by the subject and perhaps may nevertheless interfere with complete relaxation. The jerk may be diminished during the noise, as occurs in partial relaxation, and disappear when the radiator is turned off so that the room becomes quiet. In general, noises like whistling or shouts of passing students, or the starting of an automobile outside the window tended to interfere with relaxation. They tend to arouse reflexes which the subject has to relax away, if he is to be successful.

Discussion. It is important to note that the relaxation used or aimed at in these tests is more than that secured in the usual studies of the knee-jerk. It is not sufficient to have the subject sit down or recline. Under such conditions the knee-jerk will of course be present, as recently reported by Lee and Kleitman (1923).

As judged by the knee-jerk, there is great variation in individual subjects in their ability to retain a sustained relaxed state even after once secured. But objective as well as subjective criteria bear out the conclusion that the knee-jerk decreases *pari pasu* with increasing relaxation. The diminution of the knee-jerk to zero thus furnishes a simple objective criterion of the relative completeness of the relaxation.

Our results may explain the absence of the knee-jerk in a small percentage of healthy persons, when no reinforcement is used. Lombard states that Berger found it absent in 1.56 per cent of 1409 such instances. Some of these individuals at the moment of examination may be too phlegmatic or relaxed for the knee-jerk to appear without reinforcement.

Using the vasticrureus muscle in the spinal cat, Veits (1920) describes a method "to decrease its tonus until finally a point was reached where no

reflex was obtained." "It was observed that it was not possible to obtain a knee-jerk in an absolutely toneless muscle." This observation is in harmony with our own.

RÉSUMÉ AND CONCLUSIONS

The effect of extreme relaxation (without sleep) was tested with seven individuals who, (exceepting A. J. C.) had received prolonged training in progressive relaxation, with the following results:

1. The knee-jerk decreases parallel with advanced relaxation.

2. In some subjects a degree of relaxation was attained in which the knee-jerk was abolished without the individual going to sleep.

3. There is usually a "hang-over" of the effects of extreme relaxation, the jerk tending to remain diminished even after the subject is instructed to cease relaxing, and only reinforcement or moving the leg brings the response back to normal.

BIBLIOGRAPHY

BOWDITCH AND WARREN. 1890. J. of P., xi.
ERB. 1875. Arch. f. Psychiatrie, v. 803.
JACOBSON. 1920. New York Med. Journ., cxi, 419.
1921. Journ. Nerv. Ment. Dis., liii, 282; Ill. Med. Journ., xxxix, 243.
1924. Journ. Nerv. Ment. Dis., lx, 568.
1925. Amer. Journ. Psychol., xxxvi, 73; This Journal, lxxii, 387.
LEE AND KLEITMAN. 1923. This Journal, lxvii, 147.
LOMBARD. 1887. Amer. Journ. Psychol., i.
MITCHELL AND LEWIS. 1886. Trans. Assoc. Amer. Phys., i, 11.
PIÉRON. 1913. Le Problème Physiologique du Sommeil, Paris.
TUTTLE. 1924. This Journal, lxviii, 345.
VIETS. 1920. Brain, xliii, 286.
WESTPHAL. 1875. Arch. f. Psychiatrie, v, 792.

VISCERO-MOTOR REFLEXES. IV

FREDERICK R. MILLER AND R. A. WAUD

From the Department of Physiology, University of Western Ontario Medical School, London, Canada

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The viscero-motor reflexes formed the subject of recent papers from this laboratory (Miller and Simpson, 1924; Miller, 1924; Miller and Waud, 1925). In the present research the properties of these reflexes were subjected to a more minute analysis by examining the effects of various kinds of stimulation applied to the visceral nerves.

Methods. The decapitate cat of Sherrington (1909) was again employed, the decapitation being performed under profound anesthesia. The superior mesenteric nerve-plexus was chosen for stimulation by single induction shocks or the faradic current. The nerves, isolated by careful dissection along the superior mesenteric artery, were laid on small electrodes of the shielded type. After securing the electrodes in position the abdomen was closed.

The current was supplied by a Cambridge inductorium, the primary of which was fed by Edison storage cells; a rheostat and ammeter were included in the primary circuit. The amperage of the primary current and the position of the secondary coil are indicated in the different experiments. In the case of the faradic current the reading of the ammeter was taken with the interrupter in action.

For the delivery of single break currents of uniform intensity a pendulum break key was used in the primary circuit; this key was constructed so that, at the instant of opening, a signal-magnet was actuated. Summation of induction shocks was usually effected by the use of a Lewis rotary contact key (made by C. F. Palmer, Ltd., London, Eng.), which supplies break currents at rates which may be varied at will. In a few instances summation of make and break shocks was accomplished with a simple key.

Records of muscle contractions were obtained by the use of a crank-lever connected by a thread with the tendon of the muscle. In the case of the limbs the other muscles were rendered functionless by division of their tendons or by section of their nerves.

Reactions of abdominal muscles. A single break shock of moderate intensity applied to the superior mesenteric nerves elicits a short, sharp

TABLE 1

STIMULATING CURRENT	PRIMARY CURRENT	SECONDARY DISTANCE	ABDOMINAL WALL	HINDLEGS	OTHER REACTIONS
	ampères	*mm.*			
	1	110	Nil	Nil	
Single break shock. Pendulum	1	100	Contraction	Nil	
	1	90	Contraction	Slight contraction under skin of each thigh	
	1	80	Contraction	Slight contraction under skin of each thigh	
Summation make and break shocks slowly	1	100	Contraction	Plantar flexion, flexion at ankle	
Summation make and break shocks rapidly	1	100	Contraction	Both legs drawn up over abdomen with plantar flexion and flexion at hip, knee and ankle	
Faradic	0.4*	120	Contraction	Strong, bilateral flexion over abdomen	Right forepaw moved slightly forward
	0.4*	110	Contraction	Strong, bilateral flexion over abdomen	Right forepaw moved slightly forward; neck bent ventrally

* With vibrater in action.

contraction in the abdominal muscles. The threshold of stimulation is lower than that for the reflexes in the hindlegs (see table 1). The type of contraction of the *rectus abdominis* in response to a single break shock is shown in figure 1. The abdominal viscero-motor reflex thus resembles the flexion reflex of the hindleg in its capacity for being excited by a single break shock applied to an afferent nerve (Dreyer and Sherrington, 1919).

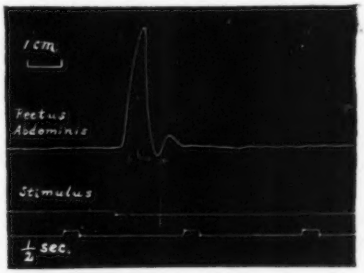

Fig. 1. Reflex contraction of *rectus abdominis* in response to single break shock applied to superior mesenteric nerves. Prim. 1.3 amp., sec. dist. 160 mm.

The result of summation of single make and break shocks is shown in figure 2. The responses exhibit some degree of tonus, which, as mentioned in a previous paper (Miller, 1924), is very pronounced in consequence of faradization of these nerves and led to the designation "viscero-tonic reflex."

Mechanical stimulation of the superior mesenteric nerves, as by a smart pinch with the forceps, yields a reflex contraction in the *rectus abdominis* similar to that evoked by a single shock.

Reactions of hindlegs. The hindlegs show reflex reactions in response to a single shock applied to the superior mesenteric nerves. As already

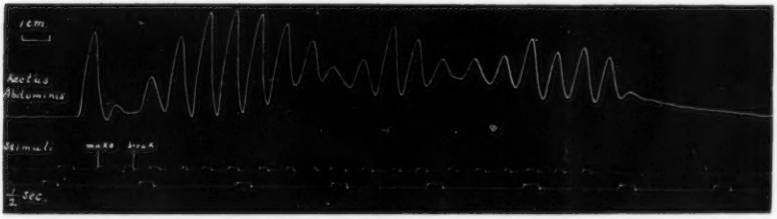

Fig. 2. Reflex contractions of *rectus abdominis* in response to make and break shocks applied to superior mesenteric nerves. Prim. 1.3 amp., sec. dist. 160 mm.

stated, the threshold is higher than that for the abdominal muscles (see table 1). With gradually increasing intensity of secondary current the first reaction elicited is often a slight contraction visible beneath the skin of both thighs, then plantar flexion, then, occasionally with a very intense single shock, flexion of legs at hip and knee and plantar flexion of feet. This latter result was obtained in one instance with a primary current of 1 ampère and a secondary distance of 80 mm.

The hindleg reflexes are very readily evoked by summation of stimuli.

TABLE 2

STIMULATING CURRENT (LEWIS'S KEY)	PRIMARY CURRENT	SECONDARY DISTANCE	ABDOMINAL WALL	HINDLEGS	OTHER REACTIONS
	ampères	*mm.*			
Single break shock	1	120	Contraction	Nil	Nil
2 break shocks per second	1	120	Contraction	Slight bilateral flexion of thighs	Slight movement of forelegs
2 break shocks per second	1	110	Contraction	Bilateral flexion of thighs	Quick clonus in forelegs
2 break shocks per second	1	100	Contraction	Bilateral flexion of thighs	Backward movement of forelegs followed by clonus
5 break shocks per second	1	100	Contraction	Bilateral flexion of thighs, plantar flexion of paws	Backward movement of forelegs followed by clonus
10 break shocks per second	1	100	Contraction	Bilateral flexion of thighs, plantar flexion of paws	Backward movement of forelegs followed by clonus
15 break shocks per second	1	100	Contraction	Bilateral flexion of thighs, plantar flexion of paws	Backward movement of forelegs followed by clonus

individually incapable of evoking any visible response in the legs. For instance, reference to table 2 will show that with a primary current of 1 ampère and secondary distance of 120 mm. a single shock yielded no visible response in the hindlegs; summation of make and break shocks, however, elicited slight but definite flexion in both legs.

Summation of shocks, individually adequate, is a still more potent means of evoking hindleg reactions. For instance, in one experiment a single break shock (2 amp. prim., 110 mm. sec.) elicited slight plantar flexion of feet; summation of 3 makes and 3 breaks yielded, together with plantar flexion, flexion at knee and hip, both legs being drawn up over the abdomen.

Similar reflex reactions, though of a more intense character, are elicited by faradisation of the superior mesenteric nerves. Thus, faradisation (0.4 amp., 120 mm. sec., table 1) yielded strong, bilateral flexion of both

Fig. 3. Posture of decapitate cat resulting from faradization of superior mesenteric nerves. Prim. 0.4 amp., sec. dist. 120 mm.

legs over abdomen (see fig. 3). Violent claw movements sometimes accompany these reactions, when they are vigorous.

Squeezing the superior mesenteric nerves with forceps yields the same type of response, namely, bilateral flexion of both hindlegs over the abdomen.

The hindleg reflexes are very susceptible to fatigue and cease completely in consequence of too prolonged stimulation; in case the reflex excitability of the animal is poor it may be quite impossible to elicit the hindleg components of the viscero-motor reflexes.

Modifications of the flexor responses above described are frequently encountered. Summation of shocks or faradisation applied to the superior mesenteric nerves frequently leads to movements of progression or kicking. In one experiment summation of 3 makes and 3 breaks per second yielded stepping and kicking. In other cases faradisation of the superior mesenteric nerves yielded stepping and kicking. Mechanical

stimulation of these nerves often yields movements of progression or kicking. At times mesenteric traction is found to elicit stepping in the hindlegs.

A further variation from the flexor response of the hindlegs is sometimes observed, extension being obtained instead of flexion or extension alternately with flexion. We had difficulty in ascertaining the cause of this type of response. We thought it might be due to differences in the intensity of the stimulating current or to varying rates of stimulation. But both these suppositions proved to be incorrect. Finally we discovered that irritation with a piece of absorbent cotton of the cross-section of the spinal cord at the level of the decapitation yields extension of the hindlegs. If faradisation of the superior mesenteric nerves is applied cotemporaneously with the cord irritation there results powerful extension of the hindlegs which, at times, is interrupted by flexions.

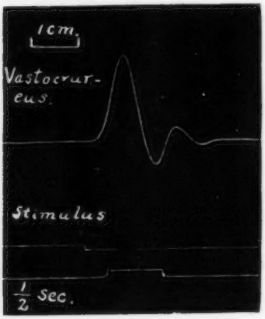

Fig. 4. Reflex contraction of *vastocrureus* in response to single break shock applied to superior mesenteric nerves. Prim. 1.3 amp., sec. dist. 120 mm.

It is probable that a further factor contributing, in some degree, to the extensor rigidity in the hindlegs is to be found in the influence of the neck reflexes of Magnus (1924), as exemplified by the bending of the neck ventrally, a condition which prevailed in many of our experiments, in which the neck was held by a string tied to a support. To test the influence of this method of fixation we tried the effect of ventral neck bending on the posture of the legs. We found that it evokes flexion of the forelegs and extension of the hindlegs; dorsal neck bending, on the other hand, evokes extension of the forelegs and flexion of the hindlegs. Both reactions occur after characteristic latency. These responses, which are identical with those described by Magnus, we find, in harmony with him, to be present in the spinal or decapitate condition.

The extension of the hindlegs sometimes yielded by faradisation of the superior mesenteric nerves is, therefore, to be explained partly by concurrent irritation of the spinal cord section and partly by the influence of the ventral neck bending reflex of Magnus. The predominant reflex response of the hindlegs to stimulation of the superior mesenteric nerves is flexion, while progression or kicking or some degree of extension may at times prevail.

Vastocrureus muscle. A single break shock applied to the superior mesenteric nerves yields contraction of the vastocrureus muscle (fig. 4). Rhythmical contractions are yielded by faradisation of the same nerves

(fig. 5). These contractions are, clearly, concerned in progression, since the opposite, intact hindleg may be observed to be performing movements of progression. Mechanical stimulation of these nerves, as by a pinch with the forceps, yields similar results (fig. 6).

A comparison of the reflex response of the vastocrureus muscle from the superior mesenteric nerves with its response in the crossed extension reflex (Sherrington, 1910), as evoked from the contralateral internal saphenous nerve, reveals the fact that in this reflex, which may be induced by single break shocks, the threshold is much lower than in the reflex from the visceral nerves. For instance, in one experiment the secondary distance for the vastocrureus reflex from the superior mesenteric nerves was 120 mm.

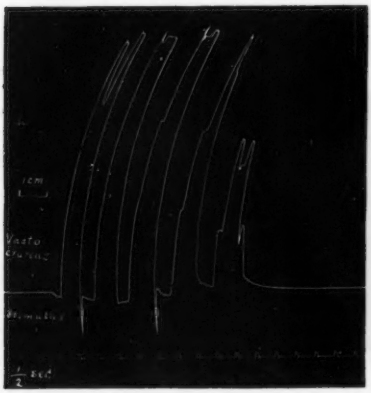

Fig. 5

Fig. 6

Fig. 5. Reflex contractions (progression) of *vastocrureus* in response to faradization of superior mesenteric nerves. Prim. 0.4 amp., sec. dist. 170 mm. Stimulation applied by opening short-circuiting key in secondary.

Fig. 6. Reflex contractions (progression) of *vastocrureus* in response to pinch applied to superior mesenteric nerves.

while for the reflex from the contralateral internal saphenous nerve the secondary distance was 180 mm. Furthermore, in the crossed extension reflex from the contralateral internal saphenous nerve the muscular response is a sustained contraction, not a series of contractions.

Semitendinosus muscle. A single break shock applied to the superior mesenteric nerves does not, in our experience, yield a contraction in the semitendinosus muscle. Summation of make and break shocks is, however, effective in evoking either single contractions, or, if more frequent, a rhythm of progression in the muscle (fig. 7). Faradisation of the superior mesenteric nerves often yields rhythmical contractions of the semi-

tendinosus, which are, obviously, those of progression, as is shown by the movements executed by the opposite hindleg (fig. 8).

Other reflex reactions. It has been observed throughout the course of this work that, during an experiment in which the superior mesenteric nerves were isolated and laid on electrodes, the abdominal walls became, continually, more and more rigid. Usually also a general increased excitability of the whole animal was noted. We were led, accordingly, to analyze some of these phenomena.

The abdominal reflex in the cat, as induced by stimulation of the skin of the abdominal wall, has not, to our knowledge, been previously described, although this reflex is well known in man (Bassoe, 1919). It may be readily excited in the normal man by stroking the skin of the abdomen with the flat end of an orange stick; the abdominal muscles contract, as may be shown by inspection or palpation.

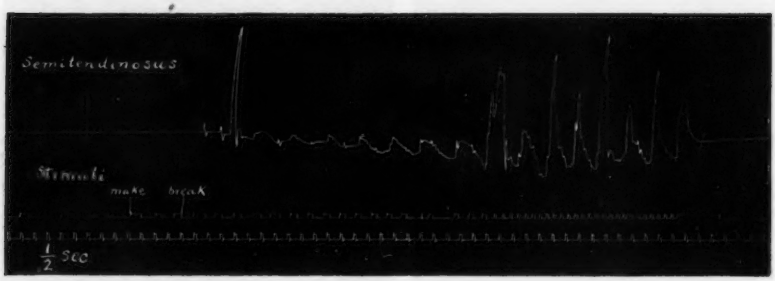

Fig. 7. Reflex contractions (progression) of *semitendinosus* in response to make and break shocks applied to superior mesenteric nerves. Prim. 1.5 amp., sec. dist. 130 mm.

We have found that the abdominal reflex may be readily elicited in the decapitate cat. For this purpose the skin is shaved on the side of the abdominal wall and the medial, ventral portions of the abdominal muscles are exposed. Stroking, with an orange stick, the shaved skin in a longitudinal direction between the lower costal margin and the iliac crest yields a very evident contraction in the abdominal muscles.

If the abdominal cavity is opened along the *linea alba* the influence of manipulation of the viscera on this abdominal reflex is found to be striking. Traction on the mesentery of the small intestine yields strong, clonic rigidity in the abdominal wall. Stroking the abdominal wall, while the mesenteric traction continues, or for a considerable time after its discontinuance, evokes much more vigorous contraction of the abdominal wall, together with general reflex effects, such as drawing up (flexion) of the ipsilateral hindleg. Pressure on the abdominal wall has a similar effect,

as also has stretching apart of the abdominal walls. Mesenteric traction thus yields powerful "facilitation" of the abdominal reflexes and the influence persists for several minutes after the intestine has been replaced in the abdominal cavity and the cavity has been closed.

It is apparent, then, that in consequence of intestinal manipulation or mesenteric traction, the abdominal muscles become intensely rigid and the abdominal reflex is much more readily elicited, i.e., shows marked facilitation. Still greater increase in the excitability of the abdominal reflexes results from faradization of the superior mesenteric nerves.

Various reactions were, at times, noted in the forelegs. It was observed, in one instance, that about 30 seconds after a rather prolonged stimulation of the superior mesenteric nerves by summation of make and break shocks, both forepaws were pressed together in the midventral line and then pushed caudally, both limbs being flexed at the wrists. At the same time the neck was bent ventrally. These reactions were repeated a number of times in exactly the same manner. They were in no wise asphyxial, since the preparation was allowed to asphyxiate afterwards and no such responses were manifested.

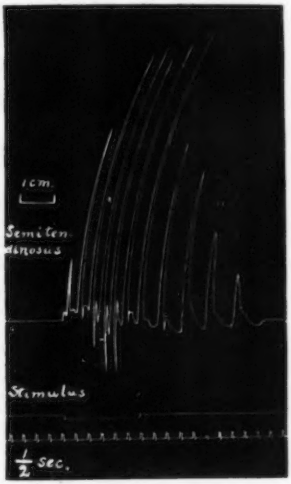

Fig. 8. Reflex contractions (progression) of *semitendinosus* in response to faradization of superior mesenteric nerves. Prim. 1.1 amp., sec. dist. 130 mm.

In another experiment one forepaw was observed to move cephalad as a result of faradization of the same nerves and at the same time the neck was bent ventrally. In yet another instance a movement caudad, succeeded by a clonus, occurred in both forelegs.

Traction on the mesentery was, at times, observed to evoke ventral flexion of the neck.

Summation of single shocks applied to the superior mesenteric nerves sometimes yields dorsal flexion of the tail; faradization usually elicits lateral lashing, sometimes with ventral flexion.

Mechanical stimulation of the abdominal skin sometimes evokes ventral flexion of the tail. To induce this reflex several strokes over the abdomen are necessary before the movement appears and then a tail movement follows each stroke. This reflex thus exhibits both summation and facilitation.

Discussion. The responses of the abdominal muscles and of the hindlegs to a single induction shock applied to the superior mesenteric nerves

are comparable to the response of the *tibialis anterior*, in the flexion reflex of the hindleg; this muscle responds by a contraction to a single break shock applied to an afferent nerve of the limb (Dreyer and Sherrington, 1919).

Pressure on the abdominal wall applied contemporaneously with visceral stimulation, such as traction on the mesentery, causes a sharp contraction of the abdominal wall. Pulling apart of the abdominal walls yields a similar response. Thus the abdominal reflex may be excited from the proprioceptors of the muscles themselves as well as from the skin. The reactions are, clearly, of the type of "myotatic reflexes" or "reflexes in response to stretch" described by Liddell and Sherrington (1924). These reflexes to stretch in a flexor muscle (*tibialis anterior*) were described in the spinal animal by Asayama (1916). Obviously, they become greatly facilitated in the abdominal muscles in consequence of afferent impulses from the viscera.

It was pointed out in a previous paper (Miller, 1924) that the purpose of the abdominal contraction was to protect a distended viscus from harmful distention or rupture. The protective mechanism is brought into play as a stretch reflex excited from the proprioceptors of the abdominal muscles.

That the hindleg components of the viscero-motor reflexes may be evoked by summation of stimuli, subliminal or liminal, has already been mentioned; in this respect these reflexes resemble others such as the scratch reflex (Sherrington, 1906) or the vomiting reflex (Miller, 1912).

The susceptibility of the hindleg viscero-motor reflexes to fatigue has already been referred to and also the ease with which they may be depressed by unfavorable conditions in the nerve-centres. These properties depend, perhaps, on special peculiarities of the synaptic connections concerned in these reflexes, though they may be due, in part, to local damage of the nerves by the electrodes and the stimulating currents.

It is evident from our results that the most "normal" responses of the hindlegs are flexion or progression. That the associated abdominal rigidity is a protective reflex was maintained by Mackenzie (1920). In a previous paper by one of us (Miller, 1924) it was pointed out that, since this abdominal contraction is elicited by distention of the stomach, its purpose is to protect the viscus against harmful distention or rupture. Admitting, then, the protective purpose of the abdominal rigidity, it is logical to infer that the hindleg components of the viscero-motor reflexes are also protective in nature. The protective purpose of flexion of the hindlegs over the abdomen appears to be that, thereby, the intra-abdominal pressure is reduced so that, for example, in case of visceral disease, with which our experimental conditions are analogous, the inflamed viscus would be subjected to less pressure.

The purpose of movements of progression, sometimes elicited in the hind-

legs, is less obvious at first sight. It is conceivable that these movements are very old, ancestrally, and that they were evolved in an aquatic ancestor, possessing a simple type of gastro-intestinal tract. In such an organism movements of progression would tend to flush out irritating or nocuous material from the digestive tract.

The protective character of the viscero-motor reflexes has just been emphasized. Arguing, as Sherrington (1900) does, that protective reflexes are associated with pain on the psychical side, it is reasonable to conclude that the viscero-motor reflexes would have, in the intact organism, pain as their psychical concomitant.

Since viscero-motor reflexes (i.e., abdominal rigidity and hindleg movements) may be induced by distention of a viscus, for instance the stomach (Miller and Simpson, 1924) and, since the reflexes constitute the pantomime of pain sensations, support is furnished for the view of Hertz (1911) that distention is the adequate stimulus for exciting the sensation of pain from the viscera. The cell bodies of the afferent visceral fibres concerned in these reflexes and in the associated pain sensations are situated in the dorsal ganglia of the spinal nerves involved (Langley, 1900; Ranson, 1921).

Reference was already made to the great increase in excitability of the abdominal reflexes (facilitation) which results from visceral irritation. This heightened reflex excitability is demonstrated in an illuminating way by the patient suffering from visceral disease; for instance, Mackenzie (1920) pointed out that in disease of the stomach the reflex contraction of the *rectus abdominis*, as elicited from the skin of the abdomen, becomes much more excitable and besides showed that the reflexogenous zone becomes greatly extended. The amplification of the reflexogenous zone for the abdominal reflex has been a marked characteristic of our experiments, in which irritation of various types has been applied to the viscera or to the visceral nerves.

SUMMARY

1. The abdominal muscles contract reflexly in response to a single break shock applied to the superior mesenteric nerves.

2. The hindlegs show reflex reactions in response to a similar stimulus. The threshold for the hindleg reflexes is higher than that for the abdominal muscles.

3. Summation of subliminal or liminal single shocks is an efficacious means of eliciting the hindleg reflexes from the superior mesenteric nerves.

4. The types of hindleg responses are: flexion over abdomen, progression, or some degree of extension. The extension is due partly to irritation of the cross-section of the spinal cord at the level of the decapitation

and partly to the influence of the neck reflexes of Magnus, which are here shown to be potent in the decapitate preparation.

5. The vastocrureus muscle contracts reflexly in response to a single break shock applied to the superior mesenteric nerves. Rhythmical contractions (progression) are yielded by faradization of the same nerves.

6. The threshold for the reflex contraction of the vastocrureus muscle in the crossed extension reflex, as yielded from the contralateral internal saphenous nerve, is lower than the threshold of the reflex contraction of the same muscle as yielded from the superior mesenteric nerves.

7. The semitendinosus muscle responds by rhythmical contractions (progression) to faradization of the superior mesenteric nerves.

8. Reflex effects of like kind to those mentioned above may be elicited by mechanical stimulation of the superior mesenteric nerves.

9. The abdominal reflex elicitable by stroking the skin of the abdomen is markedly facilitated by visceral stimulation; the reflexogenous skin zone becomes greatly extended.

10. Reflex movements of the forelegs, neck and tail are sometimes induced by faradization of the superior mesenteric nerves.

11. The reflex contraction of the abdominal muscles, resulting from stretching them while they are rigid from reflex visceral irritation, is an instance of a "myotatic reflex" or reflex to stretch.

12. The circumstance that the (protective) viscero-motor reflexes may be elicited by distention of a viscus argues in favor of Hertz's view that distention is the adequate stimulus for visceral pain.

BIBLIOGRAPHY

ASAYAMA, C. 1916. Quart. Journ. Exper. Physiol., ix, 265.

BASSOE, P. 1919. The Practical Medicine Series, viii, 5.

DREYER, N. B. AND C. S. SHERRINGTON. 1919. Proc. Roy. Soc., xc, 270.

HERTZ, A. F. 1911. The sensibility of the alimentary canal. London.

LANGLEY, J. N. 1900. Schäfer's text-book of physiology, ii, 687.

LIDDELL, E. G. T. AND C. SHERRINGTON. 1924. Proc. Roy. Soc., xcvi, 212.

MACKENZIE, J. 1920. Symptoms and their interpretation. London.

MAGNUS, R. 1924. Körperstellung. Berlin.

MILLER, F. R. 1912. Pflüger's Arch., cxliii, 1.

MILLER, F. R. 1924. This Journal, lxxi, 84.

MILLER, F. R. AND H. M. SIMPSON. 1924. Trans. Royal Soc. Canada, Sec. v, xviii, 147; This Journal, lxxii, 231.

MILLER, F. R. AND R. A. WAUD. 1925. Trans. Royal Soc. Canada, Sec. v, xix.

RANSON, S. W. 1921. The anatomy of the nervous system. Fig. 256.

SHERRINGTON, C. S. 1900. Schäfer's text-book of physiology, ii, 974.

SHERRINGTON, C. S. 1906. The integrative action of the nervous system, New Haven.

SHERRINGTON, C. S. 1909. Journ. Physiol., xxxviii, 375.

SHERRINGTON, C. S. 1910. Journ. Physiol., xl. 28.

FAT SOLUBLE VITAMINS

XXV. Further Observations on the Anti-Rachitic Action of Irradiated Animals on the Non-Irradiated when Placed in the Same Cage[1]

E. M. NELSON[2] AND H. STEENBOCK

From the Laboratory of Agricultural Chemistry, University of Wisconsin, Madison

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Huldschinsky's (1) demonstration in 1919 that ultra-violet light could be used to cure rickets established a landmark in attempts to determine the etiology of this disease. Previous to that time cod liver oil had been the only recognized agent for correcting failure of normal calcium and phosphorus deposition in growing bones.

Huldschinsky not only demonstrated that he could induce normal calcification by exposure to light from a mercury vapor quartz lamp, but also that the locus of the induced calcification was independent of the portion of the body irradiated. Hess and Unger (2) confirmed the work of Huldschinsky and showed further that sunlight was capable of effecting the same curative action on rickets as light from a mercury vapor quartz lamp. Powers and co-workers (3) showed conclusively that sunlight was also effective in preventing experimental rickets in rats. A rational explanation for the seasonal variation of rickets was thereby obtained as variations in the intensity of the sun's rays during the year are quite obvious.

In 1922 Hume (4) and Goldblatt and Soames (5) simultaneously showed that rats kept on a diet which was satisfactory in every respect except that it contained no fat-soluble vitamins did show a growth response if exposed for a short period each day to ultra-violet light. Hume postulated that the increased growth of the irradiated animals was due to a liberation of vitamin A. Steenbock and Nelson (6) confirmed these observations of increased growth induced by irradiation with ultra-violet light and showed further that the same degree of growth response could be produced by introducing aerated cod liver oil into the ration. As this aerated cod liver oil had been freed from vitamin A according to the method of McCollum (7), —which does not destroy the anti-rachitic factor completely,—they con-

[1] Published with the permission of the Director of the Wisconsin Agricultural Experiment Station, Madison.

[2] Mead Johnson & Company fellow.

cluded that the increase in growth observed was due to the action of the latter.

With the above facts in mind, Steenbock and Nelson then proposed a new method for determining vitamin A. They proceeded on the premises that rats on a ration free from the so-called fat-soluble vitamins, but containing all other indispensable constituents, will not maintain growth unless provided with a sufficiency of both vitamin A and the anti-rachitic factor. With the latter furnished by irradiation of the animals, failure of growth must then necessarily be due to lack of A. It follows that the vitamin-A content of a food material would be inversely proportional to the amount of it necessary to induce normal growth. With A abundantly provided in the ration this method was also suggested for the determination of the anti-rachitic factor by establishing the level of food intake necessary to make irradiation dispensable.

Jones, Steenbock and Nelson (8), in attempting to use this method for comparing butter fat and cod liver oil for A and anti-rachitic content, found that when irradiated and non-irradiated rats were kept in the same cage, the non-irradiated were stimulated in their growth by the presence of the irradiated.

Nelson and Steenbock (9) have already published data indicating that the explanation for this is to be sought in the anti-rachitic activation of certain compounds excreted or secreted by the irradiated animals and subsequently consumed by the non-irradiated.

Originally such an explanation did not look tenable to them because the animals were kept on wire screens which allowed all excreta to drop out of the reach of the animals, but later data made them change their opinion. Evidently the amounts of these activated constituents which have to be consumed to produce an effect are almost infinitesimal. Such amounts as may contaminate the screens are entirely sufficient.

With the possible rôle of the ingestion of these activated compounds established, it still remained to prove whether or not irradiated animals might not still give off radiations physiologically active in the same capacity. Credence was given to such a possibility, through the contentions of Kugelmas and McQuarrie (10) that compounds anti-rachitically active give off radiations affecting a photographic plate through quartz.

To obtain data on this point experiments were carried out with young rats from our stock colony. In these, some members of different litters were irradiated and kept with others not irradiated in our standard rat cages measuring 2 feet by 2 feet by 20 inches high. These cages were as usual provided with false bottoms of wire screen, 3 meshes to the inch, to prevent consumption of excreta in bulk. Other rats irradiated and non-irradiated from the same litters were kept segregated in four special cages of the construction shown in figure 1. These special cages consisted of

galvanized iron hoops 2 inches high and 10 inches in diameter, provided with screen bottoms and removable screen tops of one-third inch mesh. As the illustration shows, these cages were set up, arranged in units of two, separated by one-half inch metal strips to prevent contact.

The object of these special cages was to confine irradiated and non-irradiated animals as close to one another as possible without actual contact. Four litters of 6 rats each from 26 to 28 days old weighing from 56 to 68 grams were distributed uniformly among the 6 different groups of the experiment so that each group contained representatives from each litter. The controls were kept in the 2 standard cages—each containing 2 irradiated and 2 non-irradiated animals. The others were kept in the

Fig. 1

4 cages of the 2 units described. In one unit the irradiated animals were confined in the upper compartment and in the other in the lower.

All the rats were fed on one of our rickets-producing diets known as ration 2966. This was found superior to the McCollum or the Sherman-Pappenheimer diets as described in another publication (11). It consists of yellow corn 71, wheat gluten 20, gelatin 5, calcium carbonate 3, sodium chloride 1.

The ultra-violet light used was that produced by a Cooper Hewitt quartz mercury vapor lamp of BY type run at 50 volts and 4 amperes. The irradiated rats were exposed to this for 10 minutes daily except Sunday at a distance of 2 feet in small wire cages.

The results of these experiments at the end of 5 weeks are tabulated in table 1. In the table the caption "symptoms" refers to the stiff, awkward,

shambling gait which is distinctive of rickets in rats. When the rats are afflicted with rickets they are always very docile, hardly ever venturing to climb the sides of our cages. Their wrists are also distinctly enlarged. Histological examination of radii and ulnae was made by staining with silver after splitting with a scalpel and later after paraffin section.

The table shows in the first place, that when irradiated animals are kept in the same cage with non-irradiated animals, rickets will not develop in any of them even though they are on a diet on which rickets can be produced. In the second place, it shows that this anti-rachitic action is not brought about through the medium of secondary radiations given off by the irradiated animals. This is evident from the fact that anti-rachitic

TABLE 1

The Influence of irradiated upon non-irradiated rats on a rickets-producing diet

TREATMENT OF GROUPS	ALLOCATION TO CAGES	NUMBER OF RATS	SYMPTOMS AFTER 5 WEEKS	HISTOLOGICAL EXAMINATION OF RADII AND ULNAE	GROSS APPEARANCE OF RIBS
Lots 3052 and 3055:					
Irradiated	Confined together	4	None	Normal	Normal
Non-irradiated		4	None	Normal	Normal
Lots 3050 and 3051:					
Irradiated	Upper cage	3*	None	Normal	Normal
Non-irradiated	Lower cage	4	None	Normal	Normal
Lots 3053 and 3054:					
Irradiated	Lower cage	4	None	Normal	Normal
Non-irradiated	Upper cage	4	Rachitic	Rachitic	Beaded

* One escaped.

action was exerted only when the irradiated animals were confined above the non-irradiated. As the latter in this case had access to traces of excreta voided by the irradiated animals one is forced to the conclusion that the anti-rachitic action is effected through that medium. This emphasizes the important rôle that small amounts of activated compounds may assume because in no instance did the cages become filthy. Only traces of the excreta adhered to the screens as they were voided—dropped through the top and bottom screen of the cage. Apparently the animals in the lower cages as they walked on the screens contaminated their paws with excrement which in the course of making their toilet and picking up of food was subsequently consumed.

SUMMARY

No evidence was obtained that irradiated rats give off radiations capable of exerting an anti-rachitic effect upon their neighbors. Additional evidence was obtained that the anti-rachitic action of irradiated animals placed in the same cage with non-irradiated is effected through the medium of traces of excreta consumed. It follows that an animal irradiated with ultra-violet light has constituents in its body affected in such a manner that they become anti-rachitically active.

BIBLIOGRAPHY

(1) HULDSCHINSKY: Deutsch. Med. Wochenschr., 1919, xlv, 712.
(2) HESS AND UNGER: Proc. Soc. Exper. Biol. and Med. 1920–21, xviii, 298; Journ. Amer. Med. Assoc., 1922, lxxvii, 39; Journ. Amer. Med. Assoc., 1922, lxxviii, 29.
(3) POWERS, PARK, SHIPLEY, MCCOLLUM, AND SIMMONDS: Journ. Amer. Med. Assoc., 1922, lxxviii, 159.
(4) HUME: Lancet, 1922, ii, 1318.
(5) GOLDBLATT AND SOAMES: Lancet, 1922, ii, 1321.
(6) STEENBOCK AND NELSON: Journ. Biol. Chem., 1923, lvi, 355.
(7) MCCOLLUM, SIMMONDS, BECKER AND SHIPLEY: Bull. Johns Hopkins Hosp., 1922, xxxiii, 229.
(8) JONES, STEENBOCK AND NELSON: Journ. Met. Research, in press.
(9) NELSON AND STEENBOCK: Journ. Biol. Chem., 1925, lxii, 575.
(10) KUGELMAS AND MCQUARRIE: Science, 1924, lx, 272.
(11) STEENBOCK AND BLACK: Journ. Biol. Chem., 1925, in press.

THE MUSCULAR REACTIONS OF THE MAMMALIAN VENTRICLES TO ARTIFICIAL SURFACE STIMULI

CARL J. WIGGERS

From the Physiological Laboratory, Western Reserve University, School of Medicine, Cleveland, Ohio

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The muscular reactions of the mammalian ventricles to localized artificial stimuli have not been studied with the degree of precision that the subject merits. This is due partly to the fact that, until recently, we have possessed no graphic appliances capable of recording the ventricular contractions in an accurate manner. The details of the premature contraction curve, the latent period, the extent of the refractory phase, the physiological conditions affecting it, the factors determining effectiveness or ineffectiveness of premature beats, etc., can be accurately analyzed only when a contraction curve is recorded on which the beginning and end of systole are clearly indicated and on which the separate phases of systole and diastole are clearly demarcated as well. Such a record is supplied by the optically recorded intraventricular pressure curve supplemented for special purposes by similar pressure curves from the auricle and aorta (Wiggers, 1921).

Experimental procedures. The heart was exposed in dogs under morphine and chloretone anesthesia and after the institution of artificial respiration. Whenever possible, the pericardium was left intact and small slits were made for the insertion of the optical manometer and the application of electrodes. One optical manometer was inserted into the left ventricle and another was introduced into the aorta so that the cannula was placed just outside of the semilunar valves. For special purposes, the experiments were varied by recording the second pressure curve from the other ventricle or from the left auricle. The theory, construction and application of these instruments as well as the nature of the optical curves recorded and the criteria that they contain for determining the consecutive phases of the heart cycle have been previously described (Wiggers, 1914, 1921 a, b, 1924). A reasonable familiarity with these communications is essential in order to follow the data and conclusions presented in this paper.

Induction shocks were applied to the left ventricle by means of two small hook-electrodes, well insulated except for their ends. These were

inserted through small slits in the pericardium so that the cardial contacts were not more than 2 to 3 mm. apart. The time of stimulation was signaled on the photokymograph by a carefully adjusted and responsive signal magnet introduced into the primary circuit. The lag of the signal for break shocks as compared to the stimulus of the secondary coil was 0.0063 in an earlier series of experiments and 0.009 in a later series. This method of signaling was quite adequate for many phases of this investigation which did not concern themselves with the finest details of time relations. In the later experiments, however, electrocardiograms (usually lead II) were also recorded simultaneously in order to make quite certain that the resulting mechanical reactions were due to stimuli initiated at the same focus. This, it may be parenthetically added, is generally, but by no means always, the case when shocks are applied in this manner to the ventricle. Such records as a rule show definitely and directly the exact moment at which the induction shock is applied.

The introduction of stimuli at desired moments of systole and diastole in the rapidly beating mammalian heart is not a simple matter. While experience soon showed that the "trial and luck" method of endeavoring to introduce break shocks by manual opening and closing of a key was not in itself satisfactory, this method was nevertheless used in many experiments to supplement automatic devices. In not a few experiments gaps in the sequences of stimulation were thus fortunately filled in.

A considerable number of principles and appliances suggested in the literature for automatically timing stimuli were tried. Many proved entirely inadequate and all had some disadvantage when used in connection with optical registration. The two methods which proved most satisfactory were the following:

Method I. A Hürthle membrane manometer in which the lever was replaced by a platinum wire 5 cm. long, was inverted and connected with a carotid artery low in the neck. As the pressure within the inverted manometer increased the wire descended and in so doing dipped into a small cup of mercury, thus closing a circuit through the primary coil of the inductorium. As the pressure fell in the manometer, the lever rose, left the cup and broke the circuit. This breaking time, accomplished by lifting the lever from the cup of mercury could be finely graded through the adjustment-screw determining the lever position. By periodically closing a telegraph key also in the circuit, say every fifth beat, a stimulus was periodically applied at the same moment during every natural fifth beat of the heart. This method was useful in comparing the effects of stimuli applied to different portions of the ventricle but at corresponding moments of systole or diastole. Furthermore, it was possible gradually to advance or retard the moment of excitation by adjusting the dip of the platinum lever. In fortunate experiments, it was found possible

in this way to shift the time of stimulation from the middle of systole to the end of diastole, provided of course arterial pressures remained quite constant.

The procedure had the disadvantage, however, that the intensity of the shocks were not quite equal and that make shock could not be short-circuited conveniently. As a rule, therefore, the strength of induction shocks was so adjusted that shocks on the make were subminimal and the break shocks alone stimulated. In doing this, however, the effects yielded by the strongest shocks could not be tested.

Method II. The second method is based on the interference of natural and artificial stimuli. If artificial break stimuli are generated either by a Guthrie automatic stimulator or a Lewis rotatory stimulator at a rate which is exactly that of the heart and if further only every fifth shock is automatically allowed to stimulate the ventricle, then excitation falls exactly at the same moment of the cardiac cycle during each fifth beat. In this way comparative studies of stimuli applied at the same moment of the cardiac cycle but at different points can also be conveniently made.

When it is desired to advance or retard the moment of excitation in a consecutive series of artificial excitation it is necessary to have the natural and artificial rates slightly asynchronous. The precise tempo of the artificial stimuli required in order to advance excitation during every fifth beat by a desired amount may be exactly calculated from the formula

$$R' = \frac{60\,N\,R}{60\,N + X\,R}$$

where N represents the frequency of artificial stimulation expressed in terms of normal heart beats, R is the rate of the heart per minute, X, the interval by which it is sought to advance the stimulus each time that it is applied and R' is the frequency of artificial shocks. Thus, if it were desired to excite the heart every fifth beat, N would equal 5; and if further it were desired that each such excitation be advanced by 0.02 of a second, then X would equal 0.02. As a matter of fact, a satisfactory advance of about such an interval is obtained by merely keeping the rate of the break shock stimulator about one or two beats below that of the heart and by an automatic arrangement allowing only every fifth stimulus to reach the ventricle.[1]

The nature of premature contractions. In evaluating the reactions of cardiac muscle to artificial stimuli it has apparently been tacitly assumed that the amplitude of the recorded contraction is a criterion of the contractile power of which each unit of muscle tissue is capable and that it

[1] After the manuscript had been sent to the publishers my attention was called to the fact that a similar principle had been employed by Brücke, 1922, Zeitschr. f. Biol., lxxvi, 213.

may therefore be used as an index of the state of cardiac irritability at the time of excitation. Similarly, the duration of the contraction process is utilized as an indication of the modifications which the contractile process itself undergoes. Before such assumptions can be made, however, especially in the case of the mammalian ventricles, it is necessary to be quite certain that modifications in the spread of the excitation wave do not alter the magnitude or duration of the contraction process which the ventricle as a whole undergoes.

We may begin our analysis of this question by the presentation of two series of curves (figs. 1 and 2).

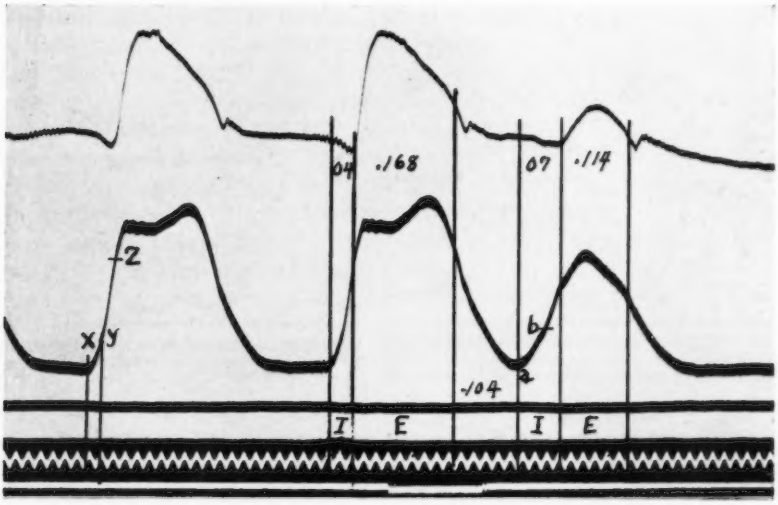

Fig. 1. Aortic (upper) and left ventricular (lower) pressure curves showing two normal beats followed by an effective premature contraction. Showing nature of the premature contraction curve. Isometric contraction phase, *I*, prolonged due to unusual lengthening of initial phase, *a-b;* ejection phase, *E*, shortened; total systole reduced. Time, 0.02 second.

Figure 1 illustrates typical effects produced by effective[2] premature contractions on the intraventricular and aortic pressure curves. A comparison of the premature (third beat) and normal beats reveals a number of differences: In the former, the isometric contraction phase is prolonged, the gradient of the pressure rise, particularly in the early portion, is

[2] The term "effective" is used in this paper to describe those premature contractions which cause an expulsion of blood into the aorta, in contradistinction to "ineffective" which characterizes those systoles that do not produce an opening of the semilunar valves or systolic discharge.

much slower and the ejection phase is abbreviated to such an extent that the total systole becomes shorter. Aside from the fundamental significance of these observations, to be analyzed presently, they are important from the dynamic viewpoint for they show that the smaller systolic discharge of premature beats is not due entirely to the smaller pressure energy developed within the ventricle but is in part attributable to the reduced phase of systolic ejection.

This abbreviation of systolic ejection and the consequent shortening of total systole accords with dynamic laws laid down by the writer and his associates (Wiggers, 1921 b, Wiggers and Katz, 1922) viz.—that the degree of diastolic filling and the initial tension not only modify the volume of systolic discharge but the duration of the ejection phase as

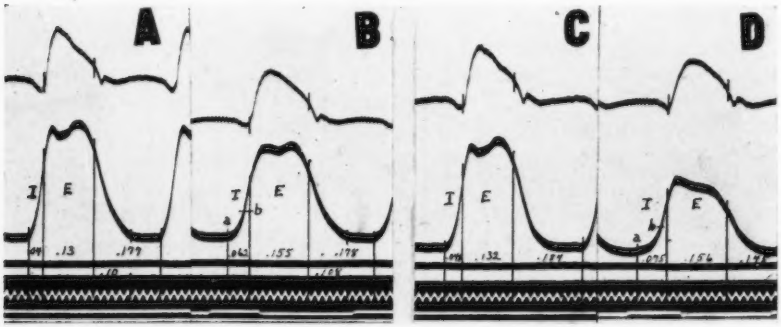

Fig. 2. Four segments of aortic and left intraventricular pressure curves. A-C, normal excitation; B-D, systoles evoked during vagal inhibition of ventricle by artificial break shocks applied at same rate as natural beat. B, stimuli applied at left apex; D, stimuli applied toward left base. Note prolongation of isometric contraction phases *I*, and slower initial rise, *a-b*, also prolongation of ejection phases *E*. Time, 0.02 second.

well; the smaller the diastolic filling, the shorter the ejection phase of effective beats becomes.

It is therefore evident that if we are to gain a full insight into the true nature of premature contractions it is further necessary to compare natural and artificially excited beats under conditions where initial pressures and diastolic filling intervals are equal.

Such a comparison is illustrated in the records of figure 2. In these experiments a series of normal beats was first recorded (segments A and C), then after complete stoppage of the heart through vagal stimulation, artificial break shocks were applied to the left ventricle either by a Guthrie automatic stimulator or by the Lewis rotatory interrupter at rates corresponding to the normal. Segment B shows beats developed when the

left ventricular surface was stimulated at the apex, segment D shows the resulting curves when stimuli were similarly applied near the left basal surface.

Under such conditions of artificial stimulation, the maximal intraventricular pressures still remain lower than normal; the systolic discharge, as evidenced by the amplitude of the aortic pressure curves, is reduced but systole as a whole is decidedly *lengthened* (e.g., cf. 0.17 and 0.217 second; 0.18 and 0.231 second). Both the phase of isometric contraction and of ejection partake of this prolongation. It should be noted, however, that at initial pressures equivalent to normal the isometric contraction phase is less prolonged than when effective systoles occur earlier in diastole, as in figure 1. Furthermore, the reduction of the maximum intraventricular pressure and of the systolic discharge below the normal level is less extensive.

It is obvious that the altered character of premature beats interposed between natural ones is in part determined by dynamic effects which are the direct consequence of an incomplete ventricular filling; but in part also by other peculiarities in the nature of the muscular contractions. It is with the latter that we are now concerned.

Cause of inherent differences in the muscular contractions. The most outstanding changes in the intraventricular pressure curves when artificial stimuli are applied to the inhibited ventricle at intervals equivalent to the natural rate, are the reduction in the height of the pressure curve and the resulting decrease in systolic discharge as evidenced by the reduced pulse pressure. Several possibilities must be considered as causes of these significant changes:

1. They may be due to depressant effects exerted by the vagus nerves on the ventricular contraction process. This suggestion, however, is invalidated not only by the lack of existing proofs that the vagus terminals reach the ventricular musculature in mammals (Drury, 1923) but is clearly disproven by the observations that similar induction shocks applied to the left auricle during vagus stoppage do not produce such changes in the intraventricular pressure curves. On the contrary, as shown in the segments of figure 3 the ventricular contraction processes remains absolutely normal.

2. The possibility that they may be due to a decrease in initial tension following abolition of auricular contractions during vagus stimulation must be considered. In segment D of figure 2 this is indeed the case and probably contributes somewhat to the smaller amplitude in this instance. In many of the records, however, the initial pressure remains unaltered as in segment B of figure 2 while in the majority it was actually elevated probably in consequence of the reduced discharge and incomplete emptying of the ventricle; nevertheless, the pressure maximum was lower.

Reduction in the initial tension is obviously not the fundamental cause of the depressed amplitude.

3. The possibility that the late contraction of the papillary muscle or the absence of auricular contraction may interfere with the proper closure of the A-V valves and thus cause a functional regurgitation is a remote possibility. Such regurgitation may, however, be definitely recognized in left auricular pressure curves (Wiggers and Feil, 1922). Careful studies of such records fail to give any indication of any change in intra-auricular pressure, suggestive of regurgitation.

4. The smaller amplitude may be due to the fact that not all fractions are excited or that in the abnormal spread of the impulses they are excited in an order or at times which differ appreciably from the normal. We

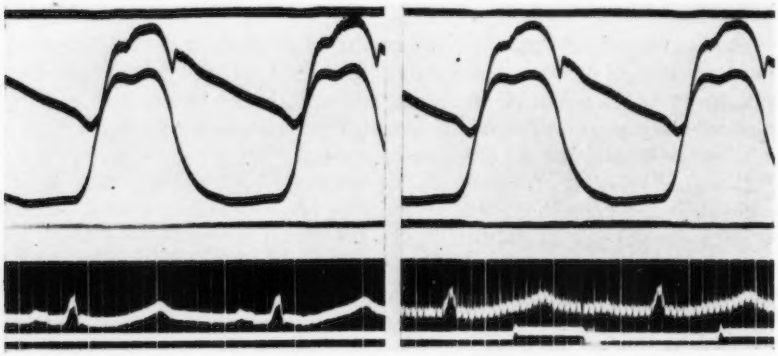

Fig. 3. Two segments of aortic and left intraventricular pressure curves together with electrocardiogram, lead II, showing that vagal stimulation has no effect on the nature of ventricular contraction. First segment, normally excited ventricle; second segment, heart inhibited by vagus stimulation and left auricle stimulated by break shocks at same rate as previous natural rhythm. Time, five divisions = 0.052 second.

must therefore consider briefly the influence which the normal and abnormal spread of impulses has on the nature of the contraction processes as revealed by intraventricular pressure curves, for the intraventricular pressure curve is a resultant of the contraction and relaxation forces operative from moment to moment. The studies of Lewis and Rothschild (1915) indicate that normally impulses are so rapidly disseminated over the His-Tawara branches and their arborization that but small time-differences exist in the excitation of the interior layers of the ventricular muscle. Owing, however, to the slower progress through the musculature of the ventricular wall and its varying thickness Lewis (1916) estimates that approximately 0.035 second is required for the excitation of the entire left ventricle of the dog (fig. 4 A). If this mode of excitation is correct

it probably follows that, when the ventricle is normally excited, ventricular systole does not begin with an absolutely synchronous contraction of all ventricular fractions; on the other hand, it probably starts as a summation of serial fractionate contractions. It is indeed logical to interpret the changing gradient of the pressure increase which takes place during the isometric contraction phase in this way. As is well shown in the normal records of figure 1 the pressure begins with an initial slow rise *X-Y* lasting from 0.02 to 0.035 second which continues as an abrupt straight rise of pressure until the semilunar valves open *Y-Z*. Assuming equivalent latent periods for all ventricular tissue it is not illogical to attribute this early rise of pressure to the serial summations of the fractionate contractions as the contraction process spreads through the ventricular wall in the wake of the excitation wave. However, when all fractions have entered the contractile state they produce the rapid elevation *Y-Z* during the remainder of the isometric contraction phase. By comparing the points indicated by *X-Y* in figures 1 and 4 A this conception of the significance of the initial rise is perhaps clarified.

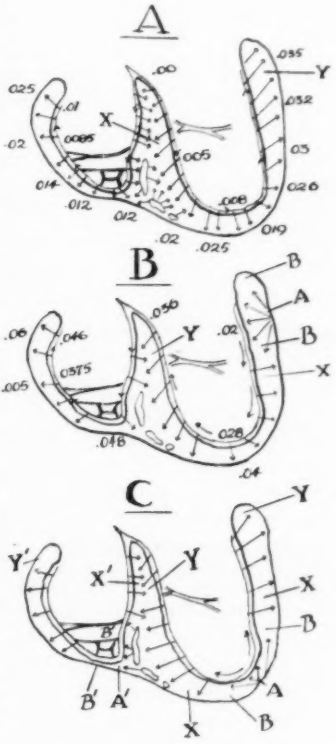

Fig. 4. Three diagrams, adapted from Lewis, showing *A*, probable normal excitation and contraction sequence; *B*, probable sequence in case of stimuli applied to the left base; *C*, probable sequence in case of stimuli applied to left apex. Points in ventricle wall labeled *A-B* indicate probable areas of localized fractionate contractions; *X-Y* points excited by Purkinje routes but contracting serially.

A careful inspection of some thirty records where artificial contractions were induced showed without fail that this early phase of slower contraction is prolonged and that the tension develops more gradually (fig. 1 beat 3, *a-b;* fig. 2 B, *a-b*). Expressed in another way, the pressure developed during equal intervals after the inception of contraction is far less in the artificially than in the naturally elicited beats. As this slower development of tension undoubtedly has a pronounced effect on the ultimate maximal tension developed its significance must be more care-

fully considered. If the initial elevation of pressure in normal beats (X-Y) is attributable to the progressive summation of fractionate contractions as the impulses pass from within outward through the ventricular walls, then it is also possible that the prolongation of this slower rise in the case of artificially elicited beats may be due to the different sequence of excitation. This accords with our conception of the spread of excitation as interpreted from electrocardiographic studies.

As summarized by Lewis (1920), "the excitation wave spreads from the point stimulated and travels in every direction radially through the muscle; it also pierces the thickness of the wall and reaches the Purkinje substance; reaching this substance, it is propagated with great rapidity over the whole lining of the corresponding chamber and ultimately spreads to the opposite ventricle." It is probable therefore that in artificially elicited beats two consecutive contraction processes merge into one another, viz., *a*, a local contraction arising at the point stimulated and spreading more or less radially from fraction to fraction; and *b*, a fairly normal contraction process of the remaining ventricular muscle excited by impulses passing in reverse direction via the bundle branches (fig. 4 B). Inasmuch as the latter also starts in a fractionate manner it follows that the duration of the slow rise should be increased by an amount essentially equal to the time required for impulses to pierce the thickness of the ventricle from without inward. The most exact calculations possible indicate that this is true in the case of artificially elicited beats recurring at nearly the same tempo as the normal. Thus, rough calculations based on normal conduction values charted in Lewis' diagram in figure 4 A indicate that the time required to excite all ventricular fractions in premature beats should not exceed the normal by more than 0.02 to 0.025 second in the case of stimuli applied to the thicker basal portions of the left ventricle or 0.01 to 0.015 second in the case of those applied to the thinner apical portions. Actual calculations from seven experiments similar to that shown in figure 2 show that the increase ranges from 0.024 to 0.031 second in the case of left basal stimuli and from 0.013 to 0.023 second in the case of left apical irritants. When the prolongation is calculated, however, in the case of premature beats occurring earlier in diastole (e.g., cf. figs. 1, 5, 10) it is found to be much greater than can be accounted for in this manner; intervals of 0.05 to 0.068 second being not uncommon. Whether this signifies that the rate of conduction through the ventricular wall is actually depressed or that impulses are not conducted directly through the cardiac layers can not be answered without further investigation.

In any case, all evidence favors the hypothesis that this early slow isometric rise of pressure in premature beats is due to an initial phase of local fractionate contractions radiating from the stimulated area.

On the basis of this interpretation of sequential fractionate and unified

contractions, the prolongation of systole as a whole may be satisfactorily explained for as the unified contractions (initiated through bundle branch distribution) start later, they also continue for a longer time. Simila ly, a number of physical reasons can readily be advanced why the less synchronous contraction of the muscles composing the ventricles could account for the more gradual gradient of the rest of the isometric contraction curve and at least contribute to the reduction of the maximal tension developed during contraction (fig. 2).

Influence of the point of stimulation on the nature of response. It was noted early in the work that stimuli applied to different portions of the left ventricles at corresponding times after the onset of diastole produce reactions of quite different magnitude and contour. A careful study shows that quite consistently the greatest reactions result when the stimuli are applied over the thinnest portions of the ventricles. Thus as shown in figure 2 stimuli applied to the thinner portion of the left apex not only produce contractions that are larger than those resulting from the basal irritants given at corresponding times but the initial rise of pressure as well as the total isometric contraction phase is much shorter. The same thing is true of stimuli occurring earlier in diastole.

These observations are quite consistent with prev ous interpretations. Obviously the shorter the distance that must be transversed in order to reach the Purkinje conducting system the smaller the fractionate contraction element derived from fiber to fiber excitation and the greater the number of fractions excited over natural pathways, with the result that more vigorous beats occur.

If these interpretations are entirely correct we should further expect that artificial stimuli applied to the opposite ventricle, would evoke an impulse which is distributed to the ventricle studied, only or largely over natural routes. The effects of such stimulation are shown in the segments of figure 5. When the intraventricular pressures are simultaneously recorded from the left and right sides of the heart, the reactions to artificial stimuli differ appreciably on the two sides. In the first place the pressure within the ventricle stimulated begins to rise from 0.03 to 0.08 of a second earlier than in the other ventricle. As shown in the different segments of figure 5 the difference is less in the case of stimuli applied to the right than the left ventricle and in each ventricle somewhat less in the case of stimuli applied to the apex, than in those applied to the base. Again these differences may be largely attributed to variations in the thickness of the ventricular wall which stand in direct relation to the order of the time difference, viz., right ventricular apex, right ventricular base, left ventricular apex, left ventricular base (cf. diagrams of fig. 4).

Further inspection indicates 1, that the vigor of the beat in the ventricle opposite to the one stimulated is always proportionately greater than in

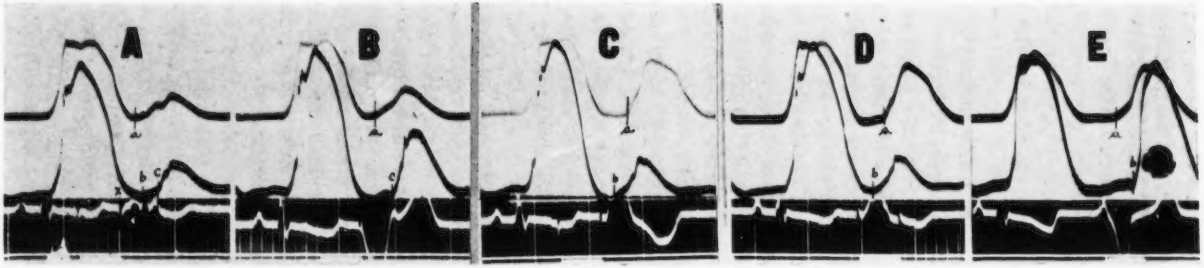

Fig. 5. Five segments of records showing left ventricular (upper) and right ventricular (middle) pressure curves and electrocardiogram, lead II (lower). Showing that pressures in opposite ventricle rise later and to relatively greater height than in ventricle stimulated. A, left basal excitation; B, left apex excitation; C, right basal excitation; D, right apex excitation; E, left apex excitation. Detailed analysis in text. *a*, beginning of left ventricular pressure elevation, *b*, slow rise of right ventricular pressure, *c*, normal rise of right ventricular pressure. Time, five divisions 0.04 second.

the ventricle stimulated; and 2, that the gradient of the pressure elevation is always much steeper there. Indeed, when the premature stimulus is given late in diastole as in the fifth segment of figure 5, the resulting contraction in the opposite ventricle often loses all the characteristics of artificially excited beats,—the initial slow elevation of pressure is absent and the isometric contraction phase is not prolonged. All of these effects indicate a spread of the impulse to the opposite ventricle over normal routes. Thus the type of spread indicated in figure 4 B somewhat exemplifies the conditions explaining the reactions of figure 5 A. When we carefully examine the records from the ventricle opposite to the one stimulated, however, we obtain many evidences that the opposite ventricle is not entirely excited in this way. This is especially true in stimuli applied near the apices of the two ventricles. Thus the anacrotic jog observed in the right ventricular pressure curves of segment B and in the left ventricular curves of segments C and D are entirely absent in smaller beats recorded when the ventricles are excited by way of the auricle. Thus, it is not improbable that, at times at least, some of the impulses pass to the opposite ventricle via the interventricular septum as indicated in figure 4 C, that is to say, some of the apex fractions of the opposite ventricle are excited by fiber to fiber transmission of impulses.

Finally, it is found that contractions elicited very late in diastole (fig. 5 E) never quite equal the normal in height. This together with the proportionately greater decrease in the pressure maximum on the side stimulated indicates that some other influence contributes to the reduced vigor of contraction. It is quite possible, especially on the stimulated side, that some fractions may escape excitation owing to an interference of stimuli in which case, much as in alternans, a certain value of contraction energy must be subtracted. Furthermore, it is not inconceivable that a certain orderliness in the mode of contraction may be necessary in order to produce a maximal effect on intraventricular pressure. The ventricular muscle is not a simple sheet of tissue but is arranged in layers of scrolls. It is quite conceivable that if, as is normally the case, either one end of the scroll or the broad base of the interventricular septum enters into contraction early it might act as a sort of fixation point for the rest of the ventricular muscle and hence cause a more effective elevation of intraventricular tension than if all portions of the scroll are excited more or less disorderly with no end definitely fixed.

Whatever part these processes play, however, it is evident that the changes in time relation and amplitude obtaining in artificially stimulated ventricles do not require the assumption of fundamental alterations in the contraction processes of the individual fractions which make up the ventricles. Consequently, it is not only necessary to keep these effects in mind in evaluating reactions from artificial stimuli but it would seem

desirable that many conclusions as to the fundamental reactions of the heart muscle, interpreted on the basis of changes in the amplitude or duration of recorded contractions, be subjected to more critical reinvestigations.

The refractory and non-refractory phases of the cardiac cycle—Previous work (for references cited cf. Tigerstedt, 1921). The results of many investigations since the pioneer observations of Schiff, Marey and Kronecker seem to have established that the normal vertebrate heart is absolutely refractory to stimuli given during the earlier portions of systole and that this is followed by a period of relative refractoriousness.

A number of observations are on record, however, that cast doubt upon the existence of such an absolutely refractory period. Thus, under the influence of toxic agents such as chloral, carbon monoxide, potassium cyanide and ether it appears that the heart is excitable during a relatively longer portion if not throughout the entire phase of systole (cf. Tigerstedt, 1921). In addition, reactions under more nearly normal physiological conditions have been reported by Carlson (1907) which indicate that in the lower vertebrates (amphibia, reptiles) stimuli applied during early systole may evoke contractions which are either higher or lower than normal beats.

A rather voluminous literature apparently also leaves unsettled the question as to whether relative refractoriousness is a late systolic or early diastolic event. Thus, referring at once to literature dealing with the mammalian ventricle, Gley (1889) interpreted his experiments on dogs and rabbits as indicating that stimuli applied late in systole are capable of prolonging the state of existing contraction to a pronounced degree. Woodworth (1903), on the other hand, concluded on the basis of experiments on contracting strips of mammalian ventricle that the muscle is absolutely refractory to the very end of systole. Cushny and Mathews (1897) again found the ventricles excitable by strong currents somewhat before the end of mechanical contractions, the reaction consisting either of a prolongation of systole or of another small contraction supported on the normal relaxation curve. Such differences of opinion must always exist in the case of mammalian hearts as long as the graphic methods do

Fig. 6. Ten segments of records showing the ventricular and electrical responses of stimuli given to left ventricle just before and just after ventricular systole. In all segments upper curves, aortic pressure; middle, left ventricular pressure; lower, electrocardiogram lead II. Arrows mark points of stimulation. A, break shock applied to left apex during P wave of electrocardiogram cause smaller premature contractions. B C D, break shocks applied to left ventricular base after completion of P produce transitional types of electrocardiograms and smaller ventricular contraction. F G H, break stimuli applied during isometric rise of pressure are without effect. I, Break stimulus during ejection phase, is without effect, J, similar break shock during ejection phase causes larger ventricular beat. All records reduced about to ⅓ original size. Time 5 divisions = 0.052 second.

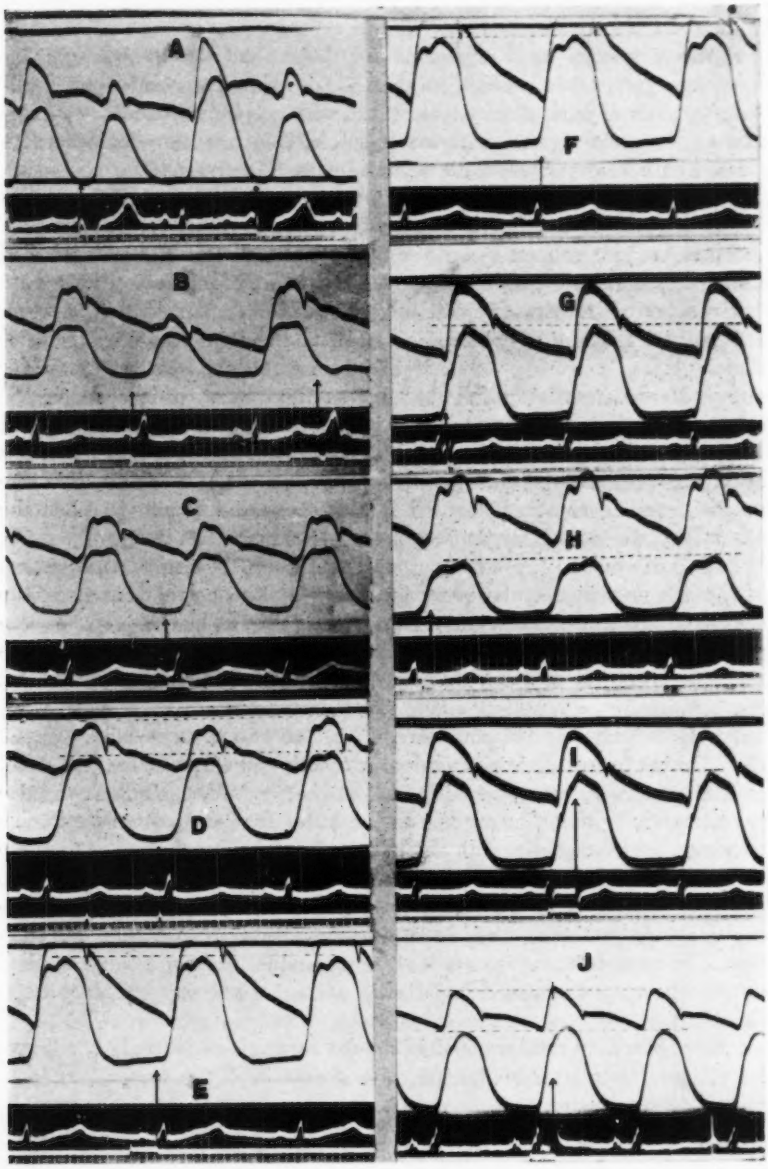

Fig. 6

not permit an accurate registration of the degree of contraction or the precise end of systole.

Effect of stimuli applied immediately before and immediately after the inception of isometric contraction. Although stimuli applied during different phases of auricular systole or just previous to ventricular contraction are in effect given during late diastole, they are more logically considered with very early systolic stimuli because the possibility exists that a stimulus applied just previous to the natural one may modify the inherent irritability and in this way affect the resulting contraction.

As shown in specimen records of figure 6 the effects of shocks applied during an interval extending from the P wave of the electrocardiogram to the onset of ventricular ejection were studied. As illustrated in figure 6 A, stimuli given during the rise or fall of the P wave and sometimes slightly later (i.e., 0.08 to 0.12 second before the rise of intraventricular pressure) are invariably followed by smaller contractions. Both the character of these ventricular pressure curves and the aberrant electrocardiograms indicate that they are obviously pure premature contractions occurring very late in diastole. The electrocardiogram shows that the normal impulse is not concerned in the excitation process. When the stimuli fall during a later portion of the P-R interval as in figure 6 B, C, D and E one of two types of reaction develops in the electrocardiograms, viz., the ventricle may be excited entirely by the normal impulse (the electrocardiogram giving typical normal impulses—fig. 6 E) or the ventricle may have a dual mode of excitation—partly from the natural impulse arriving via the A-V bundle, partly by impulses spreading from the artificially stimulated area (the electrocardiogram giving what Lewis (1920) has termed complexes of a transitional type—fig. 6 B, C, D).[3] In the former case ventricular contractions are unaffected (fig. 6 E); in the latter they are reduced in amplitude (fig. 6 B, C, D). It is quite unnecessary, however, to assume in the latter that the artificial stimulus exerts an inhibitory effect on ventricular contraction, indeed it is more probable that these effects are explained by an interference between two impulses passing over the Purkinje branches so that some fractions actually escape excitation and, as in alternans, produce a smaller contraction. In view of these results it is questionable whether stimuli applied during the time the ventricle is being excited exert any effect on irritability.

The effects of stimuli applied while the ventricle as a whole has begun its phase of isometric contraction are shown in figure 6 F, G, H. As

[3] The second electrocardiogram complex of figure 6 B shows an easily recognizable transitional complex; the second electrocardiogram complex of figure 6 C and D are chiefly due to a normal mode of excitation but have elements of the artificial spread when carefully inspected.

regards relations to the electrocardiogram such stimuli fall from a point on the descending limb of R to 0.06 second after the completion of Q R S. No instance has been found where such stimuli affect the amplitude of contraction or the pulse pressure in the aorta. As a rule, similar effects are also observed when stimuli are applied during the early phase of ejection (fig. 6 I) but in three animals an augmented intraventricular pressure and a larger pulse pressure were noted. Figure 6 J, represents a specimen of such a reaction. Such instances might be considered by many as demonstrating the non-refractory condition of early systole. Incidental evidence, however, does not make these observations entirely convincing. In the beats where this occurred other factors could not always be excluded, sometimes the initial tension was slightly higher, again the length of the preceding cycle was slightly greater but most suspicious of all, similar larger beats occasionally occur when the stimulus is applied after the maximum ejection has already been reached. Thus in figure 6 J the break shock is actually not applied until the highest systolic pressure in the aorta has already been developed, but previous to that owing to imperfect contact a series of minute and irregular shocks apparently had reached the ventricle. Even if we discount entirely the many negative observations and stress disproportionately these positive experiments it is still not necessary to interpret these supermaximal beats as evidence that the ventricles are irritable during early systole. In view of the fact that they result only when stimuli are given relatively late after the inception of contractions it would be far more logical to presuppose the additional stimulation of fractions which had escaped excitation by the normal impulse than it would be to suppose that such late stimuli could have an almost immediate effect on the irritability of muscle fractions already in the process of contraction.

We must therefore conclude that there is no really convincing evidence that the early phase of systolic contraction in the mammalian heart is non-refractory to stimuli. Experiments on lower vertebrates must be submitted to further experimentation which exclude influences of impulse interference before they can be finally accepted.

Effect of stimuli applied late in systole. The results to be reported are based chiefly on a careful analysis of twelve experiments in which one hundred and ten shocks given at various fractions of the last tenth of systole, were analyzed mathematically, the beginning of the aortic incisura being used as the criterion of the end of ventricular systole. In addition, similar effects obviously present in many other stimulations but not mathematically analyzed, support the conclusions drawn.

Experiments in which the end of ventricular systole is clearly located show definitely *that the latter portion of ventricular systole is not refractory to stimuli.* This non-refractory phase of systole comprises a variable

portion of the latter part of systole and its duration varies slightly in different animals. While the end point of reactivity could not always be determined it may be stated that in twelve experiments the longest non-refractory intervals obtainable before the end of systole were 0.045, 0.048, 0.021,[4] 0.055, 0.048, 0.0543, 0.0524, 0.069, 0.03, 0.066, 0.076, 0.105[5] respectively. That this is truly a phase of relative refractoriousness is attested by the fact that the extent of this phase is governed by the intensity of the stimulus. Thus, in experiment 324, II, a strong shock caused a reaction 0.058 of a second before the end of systole, and a moderate stimulus caused a reaction 0.032 of a second before the end of systole. A weak shock caused no response when given 0.027 of a second before the end of systole but elicited a response when the interval was reduced to 0.02 of a second.

How closely the very beginning of these non-refractory phases may be determined in fortunate experiments is shown in figures 7 and 8. In the former illustration are shown records obtained when the heart was automatically stimulated by the Hürthle stimulator (method I). As the arterial systolic pressure fell a trifle from beat to beat this operated to withdraw the platinum contact from the mercury cup a little sooner each beat. As indicated in the early portion of this record, strong break stimuli applied 0.0664 −0.0654, −0.0634 of a second before the end of systole were without effect but a definite reaction was caused by a stimulus preceding the end of systole by 0.0453 of a second. The gap is filled by the beats shown in the latter part of this record where a stimulus applied 0.0564 of a second before the end of systole was ineffective but one applied 0.0524 of a second before systole terminated caused a reaction.

On the whole it may be said therefore that under ordinary experimental conditions, the dog's ventricle remains refractory to stimuli until about 0.06 before the termination of systole. Sometimes this range is not reached and occasionally, as shown in the data above, longer non-refractory intervals (even up to 0.105 of a second) are indicated. The latter fact is also shown in the last post-compensatory beat of figure 7 and the records of figure 8. In a latter experiment the stimulus was advanced by the interference method of stimulation from the point of exact termination of systole (segment B) to 0.083 of a second before the termination of systole (segment G) with positive reactions throughout.

Careful tabulations indicate that this extension of the non-refractory phase occurs pari passu with the prolongation of systole. Thus, the longer non-refractory intervals shown in figure 8 are due to the unusually long systoles and the further extension of this period in the last beat of

[4] End point not even approximately reached in this experiment.

[5] From an experiment with an unusually long systole.

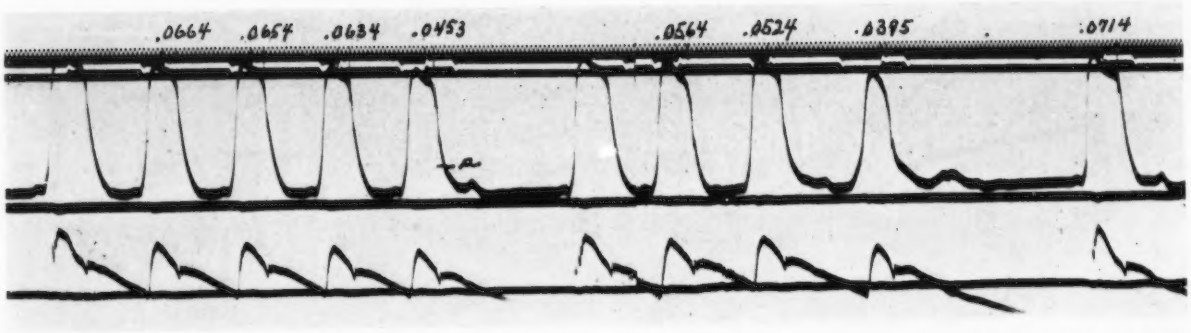

Fig. 7. Series of ventricular and aortic pressure curves. Left ventricular apex stimulated progressively later in systole. Figures above curves give intervals before end of systole. In the fifth natural beat a stimulus 0.0453 second before end of systole is effective, in the eighth natural beat a stimulus 0.0524 second before end of systole is effective. The last beat which is post-compensatory causes the extension of the non-refractory to at least 0.0714 second, due to lengthening of total systole.

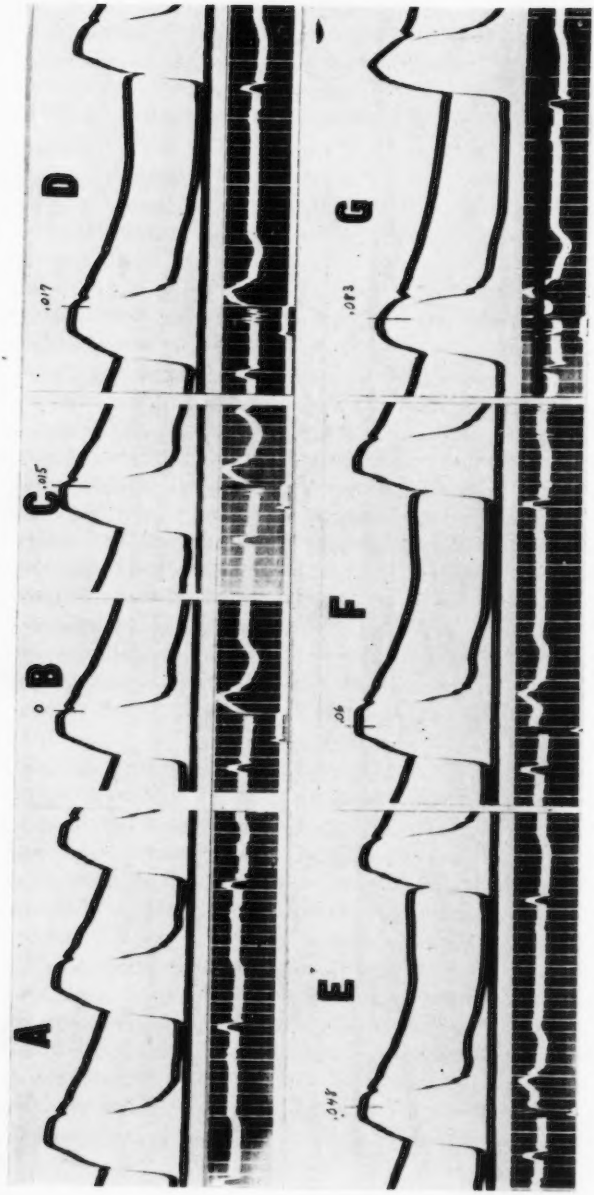

Fig. 8. Segments of records showing the effect of stimuli applied at various intervals of late systole and the nature of the resulting contraction—upper curve, aortic pressure; middle, left intraventricular pressure; lower, electrocardiogram lead II. A, normal series of beats. Figures give intervals by which stimuli were applied before end of systole. Time, 5 divisions, 0.052 second.

figure 7 is associated with the longer systole of the post-compensatory beat. Further explicit illustrations may be mentioned, however:

In experiment C 324 a strong shock applied 0.05 of a second before the end of systole evoked no response but caused a reaction 0.055 of a second before the termination of ventricular contraction when the systole length had been increased by vagal stimulation. Again in experiment C 330 XIII a stimulus applied 0.0564 of a second before systole end was negative, but one applied 0.0524 of a second evoked a contraction. Two stimuli, however, were applied during two similar compensatory beats which had their systoles prolonged by 0.03 of a second; one of these given 0.0714 of a second before termination evoked a reaction, the other given 0.0765 of a second previous to its end was negative. Similarly, when systole was lengthened independently of cycle length (e.g., by venous infusion) the non-refractory phase was prolonged. For example, in experiment C 326 a strong stimulus given 0.048 of a second before the end of systole and yielding no response caused a good reaction after systole had been prolonged by venous infusion. No definite or constant ratio between systole length and either the refractory or non-refractory period of systole as maintained by Schultze (1906) could be established. This is of course not surprising inasmuch as no reason exists why such exact mathematical relation should occur and its presence in any series of experiments must probably be regarded as accidental.

Do these reactions necessarily imply that the fractions of ventricular muscle are actually irritable while still contracting? The argument may be advanced that since some fractions normally begin their contractions 0.025 to 0.035 second before others and inasmuch as they may fairly be supposed to stop contracting earlier, it is not impossible that the response is due to the excitations of these early relaxing fractions. This interpretation is invalidated by the facts 1, that the ventricle is excitable long before we have reason to suppose that such relaxation processes are in existence (cf. non-refractory phase of 0.06 second with fractionate contraction phase of 0.025–0.035 second); and 2, that the late systolic responses are obtained when stimuli are applied to the base of the left ventricle which is undoubtedly the last portion to begin and end its contraction. The results therefore indicate that the state of refractoriousness disappears during the latter portion of the contractile stage. The fundamental significance of this change must await further investigation.

Nature of the contractions following stimuli applied during the non-refractory phase of systole. In myographic studies published by Gley (1889), Cushny and Mathews (1897) and Woodworth (1903), premature contractions are apparently never superimposed on normal curves, using this term in the sense that the resulting contraction summit is higher than

normal. Indeed Hirschfelder and Eyster (1907) explicitly emphasize that premature contractions are *always* smaller than normal beats. Graphic evidence has, however, been published by Hering (1905), Rihl (1907) and MacWilliam (1923) which apparently demonstrates that such super-position is possible. If this is the case they must necessarily result from stimuli applied before ventricular systole is over, i.e., during the non-refractory phase. Finally, the graphic curves of Gley (1889) as well as those of Cushny and Mathews (1897) indicate that while no superposition results from stimuli applied during the non-refractory phase of systole, they do cause an extension of the systole, in which they fall.

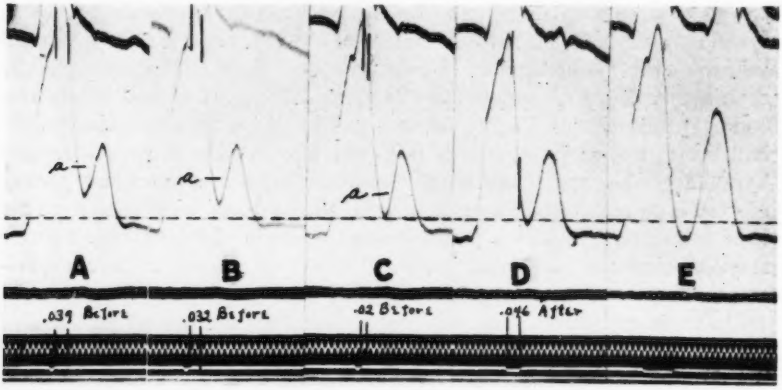

Fig. 9. Segments of aortic and left intraventricular pressure curves showing effects of break stimuli (lower marker) applied during late systole (A B C) during isometric relaxation (D) and during rapid inflow phase (E). Discussion in text. Numerals indicate intervals before or after end of systole that stimuli were applied. Time $\frac{1}{30}$ second; lower marker stimulus recorder.

A careful examination of many optical tracings of intraventricular pressure shows evidence neither of a superposition of premature beats nor of a prolongation of systole when stimuli are applied during the non-refractory period of systole. On the contrary, the contractions never begin until near the end of the isometric relaxation process. The curves of figure 7, 8, 9 and 10 illustrate the nature of the responses.

If there is any local muscular contraction during the early moments of the isometric relaxation phase, it is obscured by the predominant influence of the relaxation processes still going on, for the most careful superposition of records fails to bring out any change in the gradient of the isometric relaxation curve until the very end, where changes are merely a matter of degree. Thus, as shown at *a* in figure 9 A there may be just the merest trace of a decreased gradient or as similarly shown at *a* in

figure 7 and 10 A the change may be more obvious. Again as illustrated in various segments of figure 8, the change may amount to a distinct halt in the decline of the pressure curve.

These earlier changes are followed, either by a prolongation of the relaxation process as shown in segments D and G of figure 8 or, what is more common, by a small isometric elevation of pressure such as is shown in figures 7, 8 E, F, and 10 A, B. The greatest reactions recorded in the entire series which resulted from stimuli applied to the left apex are shown in figure 9 A, B and C.

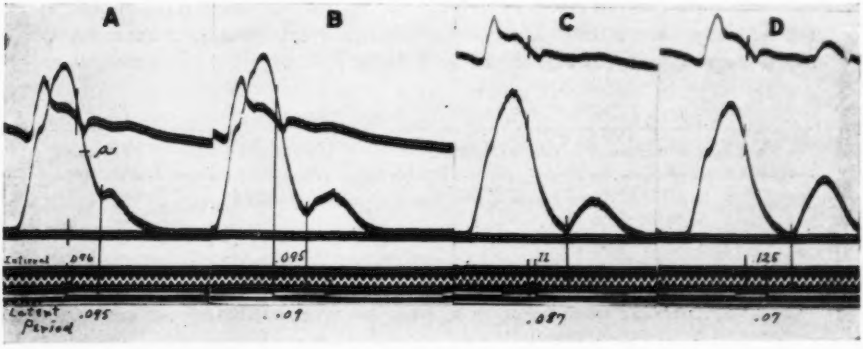

Fig. 10. Four segments of aortic and left intraventricular pressure curves showing effects of stimuli applied during late systole (A) and during isometric relaxation phase (B C) and during inflow phase (D). Numerals above tuning fork denote intervals after end of systole—observe that 0.125 second is required to produce effective beat.

Note progressive decrease in latent period (lower numerals), progressive decrease in pressure maximum and larger amplitude of upstroke in A B C. Initial tension higher in A B and C than in D but initial length less, indicating that latter fundamentally determines amplitude of reaction. Time $\frac{1}{10}$ second.

With such clear-cut results following the application of stimuli to the external surface of the ventricles during the isometric relaxation phase we can only conclude that the contrary evidence of superimposed beats and extended systoles obtained by the graphic methods must be attributed either to faulty methods of registration or to the fact that the hearts in question were decidedly abnormal.

From the dynamic viewpoint higher pressure maxima developed by superimposed beats would be quite as ineffective as though the contractions occurred when they do. Beginning before the A-V valves have opened, the ventricles have received no blood and consequently are quite unable to discharge any.

The apparent latent period. It seems to have been adequately demonstrated on amphibian hearts that the latent period becomes longer as the stimulus is applied progressively earlier in the non-refractory phases. This is corroborated in these mammalian experiments. A stimulus applied so late in diastole that it barely precedes the next natural excitation produces a contraction within 0.03 to 0.04 of a second. A stimulus applied during the isometric relaxation phase, however, requires an interval of about 0.06 to 0.08 of a second while one given during the non-refractory phase of systole causes no pressure elevation for 0.09 to 0.12 of a second.

While these results can be confirmed in records shown in various illustrations they are more readily followed in the tabulated results from a single experiment (C 326 I, II and III).

TABLE 1

TIME RELATION OF EXCITATION TO END OF SYSTOLE	LATENT PERIOD
	second
0.045 before end	0.121
0.043 before end	0.116
0.032 before end	0.118
0.03 before end	0.110
0.028 before end	0.100
0.025 before end	0.102
0.035 after end	0.08
0.092 after end	0.062
0.094 after end	0.06
0.110 after end	0.062
0.13 after end	0.06
0.15 after end	0.045

It is not necessary, however, to assume a peculiar recovery gradient of cardiac muscles during the so-called recovery period to explain this shortening of the apparent latent period. Undoubtedly even the shortest latent period includes, in addition to any adherent latency of muscle response, the time required for the spread of the excitation wave to a sufficient number of muscle units to produce a recordable response of the ventricle as a whole. The number of contracting fractions required to elevate intraventricular pressure also depends on whether the remaining fractions are at rest or in the process of relaxation; the greater the relaxing forces operative in the entire ventricle at the time of stimulation, the greater the number of fractions that must contract before the resultant of the two forces manifests itself as an elevation of intraventricular pressure. To judge from the changing gradients of the normal isometric relaxation curve the total relaxation forces in the early part of this phase

exceed those in the latter portion. Consequently, the earlier that contractions are initiated, the greater the number of contracting fractions required before the relaxation forces are over-powered and therefore the longer the apparent latent period becomes.

The fact that the early energy of these contractions can not be utilized in the elevation of pressure but is spent in counteracting the fall in pressure accounts also for the facts 1, that no initial period of gradual fractionate contraction is exhibited in the rise of the early beats, and 2, that the gradient of contraction becomes progressively steeper, the later the beats come in the isometric relaxation phase (figs. 9 and 10).

Finally, the factors concerned in extending the apparent latent period when shocks are applied during systole explain also why it is impossible for them either to prolong systole or to cause superimposed beats early in relaxation. Assuming a minimal latent period of 0.03 of a second between excitation and response in the case of a stimulus applied 0.06 of a second before the end of systole and a minimum interval of 0.035 of a second before impulses are distributed by the Purkinje branches it is obvious that no considerable number of ventricular fractions have begun to contract until systole is over, thus effectively preventing an extension of the contraction phase. Furthermore, by the time the impulse has had time to spread to the entire ventricular musculature, the ventricle has had time to relax to a considerable degree, thus resulting only in a pressure rise during the latter part of this phase.

Therefore, not only do experimental results of this investigation fail to give evidence of extended systoles or superimposed beats but a consideration of the factors concerned in the production of these contractions seem to make their existence quite impossible.

Factors determining the vigor of mammalian premature contractions. It has already been shown that owing to the fractionate onset of the premature contractions they are always smaller than normally excited beats even when the intervals between beats are the same. Among the further facts that appear to have been clearly demonstrated is the one that the amplitude of the premature contraction increases as the time elapsing since the end of the previous systole increases (for references of cf. Tigerstedt, 1921). How far these changes may be accounted for, however, by a gradual recovery of irritability and contractility and to what extent they may be due to the effect of variable degrees of ventricular filling do not appear to have been clearly separated.

As regards the influence of the time factor Woodworth (1903) has clearly pointed out that two opposing forces must be concerned in determining the conditions of the muscle for response, viz.: "the stimulating effect of a rapid succession of contractions and the recuperative effect of a long pause." As regards ventricular strips from mammalian hearts

Woodworth concluded that these opposing forces operate so that the optimum interval for stimulation is about one second or slightly less. As this interval is in excess of the time required for a normal heart cycle in the dog it is obvious that cardiac muscle is not normally beating at its best. Adrian (1920) and Wastl (1922) have shown more recently that the frog's ventricle may under certain conditions exhibit a superirritable and supercontractile phase, in this respect resembling the recovery process in nerve.

In order to evaluate the relative rôles played by possible hypercontractile phases, by the recovery interval of diastole and by changes in the degree of ventricular filling, a careful study of the contractions falling in different phases of diastole was made. Inasmuch as in the normal dog's heart there is an interval of some 0.07 to 0.09 of a second after systole before ventricular filling begins (protodiastole and isometric relaxation) it was hoped that an evaluation of the time factor might be made over a considerable interval where any resulting contraction must be of an isometric nature. Furthermore, as the super-contractile phase apparently begins soon after the relative refractory phase there was some reason to hope that evidence of it might be obtained during this phase if it were present in the mammalian heart under experimental conditions. The discovery that no contractions can be elicited until the very last portion of this phase, even by stimuli applied during late systole made this part of the inquiry far less complete than was hoped for.

The results of these experiments were consistent and are typified in the records of figures 9, 10 and 11. It is obvious that as the contractions begin later and later during the isometric relaxation phase the following changes occur: 1, the amplitude of pressure elevation measured by the vertical height of the ascending limb become larger; 2, the gradient of the pressure rise becomes steeper, but 3, the pressure summits reached tend to decrease somewhat. It is further obvious that there is a critical region just after the end of the isometric relaxation phase where small differences in the onset of contraction produce marked changes in the pressure maximum (cf. fig. 9, D, E; fig. 10 C and D). Obviously this shows clearly the additional influence of ventricular filling with the resultant stretching of cardiac muscle. The absence of contractions with amplitudes larger than those of normal beats during any phase of diastole precludes the existence of a supernormal phase of contractility. It is not quite so easy, however, to exclude the possibility of a *comparatively heightened* phase of contraction during the earliest portion of the isometric relaxation phase inasmuch as it is not at all clear whether or not the vigor of the premature isometric contractions become progressively greater as a result of the time factor of recovery. Much depends on the criterion adopted. Thus in the premature beats shown in the first segments of

figures 9 and 10 the amplitude as measured by the vertical height of the ascending limb increases but the systolic pressure maxima actually become somewhat less.

In the interpretation of records from frog hearts the former criterion has been adopted; from the dynamic point of view the highest pressure maxima would appear to be the more logical index of muscle work. According to the first criterion the muscle reactions are considered progressively stronger; according to the latter they become progressively somewhat weaker. Nor does the degree of prematurity in the excitation appear to influence the magnitude of the reactions appreciably, as long as the contractions begin during the isometric relaxation phase. A stimulus applied during the non-refractory stage of systole yields practically the same pressure maximum as one given after the isometric relaxation phase has begun (fig. 9 A-D; fig. 10 A-D).

When the incidence of the premature contraction is delayed until a small inflow has taken place as shown in the fifth segment of figure 9, a considerably greater elevation of pressure and a higher pressure maximum develops. If we examine the amplitude of contractions occurring progressively later during the period of ventricular filling, it is observed that the amplitude of contraction increases. Analysis of the cardiodynamic effects produced during these intervals indicates, however, that it is neither entirely nor predominantly due to a progressive recovery of irritability or contractility as determined by the longer time interval for recovery. That this time factor per se may be concerned to some degree may not be doubted. Thus, when at very slow heart rates following vagal stimulation premature contractions occur during a long diastasis (i.e., when no further changes in initial tension are concerned) they may be consistently slightly larger than beats coming somewhat earlier. When, however, premature contractions occur during the rapid inflow phase (fig. 11) we note at once that the amplitude of contraction increases or decreases as the initial tension in the ventricle. Therefore, I believe the larger amplitudes to be related to the time factor solely because the longer the interval, the greater the ventricular filling and initial tension. Furthermore, if for any reasons the rate of inflow increases (e.g., as during venous infusion) then the initial tension increases more rapidly and the very early beats may become much greater.

We may therefore conclude that the recuperative effect of a longer time interval, as this affects the recovery process, plays but a minor rôle in determining the progressive increase in the amplitude of premature beats beginning later and later in diastole but that changes in cardiac irritability occasioned by the increased filling and stretching of the ventricles plays the dominant rôle.

Initial length and initial tension as fundamental factors determining

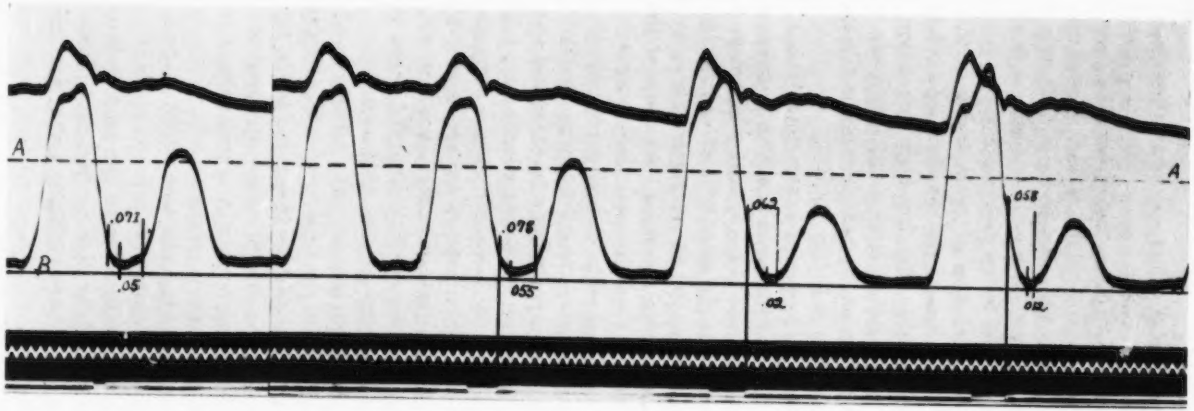

Fig. 11. Series of five break shocks given progressively earlier during the isometric relaxation phase and producing progressively smaller and earlier contractions. Showing, in addition to characteristics of contraction curves occurring at various stages of diastole, that amplitude of contraction is determined by duration of inflow phase (approximately given by lower numerals) and by initial tension (line B). Upper numerals show apparent latent interval between time of stimulation and the main elevation of pressure. Note disappearance of period of initial slow rise in last beats which come earlier in diastole. Time $\frac{1}{50}$ second.

cardiac response. Considerable discussion has arisen as to whether changes in initial length or initial tension fundamentally determine the vigor of ventricular reaction. As the subject has recently been reviewed by the writer (Wiggers, 1923) it is merely necessary in this connection to recall the results of my own experiments. In extensive series of experiments of a variable sort I have never found an instance in which actually observed or probable increases in diastolic volume failed to be associated with an increase in initial tension. I could therefore only conclude either that initial tension is directly responsible for the magnitude of the ventricular response or if the initial length of muscle fibers is fundamentally responsible for changes in vigor of contraction it is only through a previous increase in initial tension that such an increase in length can be produced (Wiggers 1921 b, 1921 d). Such experiments could obviously not determine whether initial tension or initial length is the fundamental determinant of the amplitude of reaction. It was suggested that by comparing the initial tensions at which premature contractions of different height in various phases of diastole occur, some definite relation or lack of relation might be established. In the beats occurring during isometric relaxation the initial tension and length proceed in opposite directions, the longer the fibers become during relaxation the lower the initial tension (figs. 9 and 10); during inflow on the contrary they go hand in hand (fig. 11). Owing to the lack of a clear criterion as to whether amplitude of ascending limb or the pressure maximum represents a true measure of the vigor of response it is not possible, at present, to draw conclusions from a comparison of the consecutive beats occurring during the isometric relaxation phase alone. Thus, in the first three segments of figures 9 and 10 it is obvious that the pressure maximum is definitely related to the initial tension under which beats start, while the amplitude of the pressure elevation is distinctly related to the length of the muscle fibers. When, however, the beats beginning during the isometric relaxation phase (fig. 9 A, B, C) are compared with those during ventricular filling (figs. 9 D, E; 10 C, D) it becomes obvious that neither the amplitude nor pressure maxima are determined by initial tension but rather by the state of lengthening that the fibers have undergone. Unless the time recovery factor indeed plays a far greater rôle than we have any reason to suppose from a priori considerations these results indicate clearly that, as in striated muscles, initial length is the fundamental factor concerned in determining the vigor of ventricular contractions.

It may be reiterated, however, that this in no wise compromises the position that when the cycle is of average normal duration, changes in initial length are produced solely by previous changes in initial tension. This holds true also in premature beats occurring during successive intervals of the inflow phase. As is clearly shown in the segments of figure

11, the amplitude changes correspond definitely with changes in initial tension as well as with probable changes in initial volume.

Factors determining effectiveness of premature systoles. Premature contractions may be *effective*, i.e., cause a systolic expulsion of blood; or they may be *ineffective*, i.e., fail to increase intraventricular tension sufficiently to open the semilunar valves. In the latter instance pure isometric contractions are produced. Effective systoles are recognized 1, by the production of clear cut aortic pulses; 2, by the fact that the intraventricular pressure rises beyond the point where ejection normally occurs, and 3, by the sharper pressure drop in the isometric relaxation phase indicating that contraction has not been of an isometric character. All of these criteria should be especially examined when only small pressure variations occur in the aorta for such variations may be the results of traction effects without opening of the semilunar valves and consequently without ejection being present.

Results obtained in experiments on 15 dogs were carefully studied as regards the factors determining the effectiveness of premature systoles.

Of fundamental importance is a minimal interval of ventricular filling. At first, efforts were made to calculate directly the minimal intervals of rapid inflow required in order to fill the ventricle sufficiently to produce an ejection. Inasmuch as the moment of inflow is so varying in its relation to the descending limb of the ventricular pressure curve, however it seemed the wiser policy to relate these events to the end of systole. The abbreviated data of table 2 indicate the nature of the results around critical regions. On the whole they show that an average interval of about 0.12 of a second must elapse between the end of systole and the onset of the premature contraction, the shortest interval producing effective beats being 0.098 of a second and the longest, 0.14 of a second. As the writer (1921a) has shown that two phases of diastole occur between the end of systole and the opening of the A-V valves, viz., the protodiastolic and isometric relaxation phases which together occupy from 0.072 to 0.08 of a second, this means that a minimal interval of about 0.04 of a second is required in order to fill the ventricle sufficiently to cause an ejection against normal arterial diastolic pressures. Occasionally systoles occurring 0.055 of a second after the calculated beginning of ventricular filling are ineffective. The actual interval that elapses depends, however, on the arterial diastolic pressure being somewhat less when this pressure is low (e.g., table 2, experiments C 268 and C 270 XI). It is also influenced by the height of the venous pressure, contractions becoming effective somewhat earlier when the rate of ventricular inflow is increased by venous infusions.

In accordance with these results, the interval required for effective filling is modified also by the extent to which the preceding systole has

emptied the ventricle. It is often true that a normal cycle following a post-compensatory beat is somewhat smaller than regular beats, due to the more complete emptying of the ventricle during the post-compensatory systole. In such animals the filling interval required to produce effective beats is longer in the case of premature systoles immediately after such post-compensatory beats.

TABLE 2

EXPERIMENT	AFTER END OF SYSTOLE	REACTION	DIASTOLIC PRESSURE	EFFECTIVE VENOUS PRESSURE
C 217 VI	0.108	Ineffective	73	
	0.12	Just effective	73	
C 218 XII	0.105	Ineffective	64	
	0.18	Very effective	67	
C 265 VI	0.11	Ineffective	81	
C 268 IV	0.148	Almost effective	68	
	0.178	Very effective	68	
C 268 VII	0.09	Ineffective	60	
	0.14	Very effective	33	
	0.108	Ineffective	46	
C 270 XI	0.098	Just effective	73	
	0.069	Just effective	29	
C 320	0.098	Ineffective	55	
	0.131	Almost effective	?	
C 321 XI	0.094	Ineffective	48	
	0.098	Just effective	43	
	0.132	Very effective	43	
C 322 III	0.105	Almost effective		
	0.115	Almost effective		
	0.098	Ineffective		
C 323 V a	0.108	Barely effective	57	60
	0.112	Not effective	78	83
VI a	0.122	Just effective	81	83
	0.117	Just effective	59	75
C 324 II b	0.100	Not effective		80
	0.114	Quite effective		80
C 330 XVIII	0.12	Not effective		
	0.135	Quite effective	Same	Same

Finally, there is evidence that the filling interval required to produce effective systoles may be affected by the rate of isometric relaxation. Thus, in experiment C 330, XVIII, a premature systole occurring 0.12 second after the end of systole was not effective while another 0.135 of a second proved quite effective. An analysis of the ventricular pressure curve showed that this was not entirely attributable to the differences in filling time for the isometric relaxation phase was at least 0.02 of a second less in the latter case, thus permitting ventricular filling to begin earlier.

SUMMARY

The pressure curves from the ventricles and aorta when the ventricles are normally or artificially excited are compared and analyzed, the results are harmonized with previous knowledge as to the spread of the excitation wave in the two instances and the following interpretation of the reactions is the result:

1. When normally excited via the A-V bundle and its branches the intraventricular pressure curve begins with an initial slow elevation of tension lasting from 0.02 to 0.035 second (fig. 2, *x-y*). This is now interpreted as due to the fact that not all fractions of the ventricle begin their contractions simultaneously but rather as a series of sequential fractionate contractions. The process differs from that in the auricle only as regards the greater speed of excitation and the consequently longer interval of unified contraction which follows.

2. When artificial stimuli are given to the inhibited ventricles at rates corresponding to the normal for any heart, the initial slower rise of intraventricular pressure is prolonged, the isometric contraction phase is lengthened, the gradient is not so steep, the pressure maximum is lower and the duration of systole is increased. Such changes do not indicate fundamental alterations in the properties of contractility or irritability, however, nor may they be interpreted as evidence that similar changes occur in the fundamental contractions of the separate fractions of ventricular muscle. On the contrary, they are explained by differences in the order of excitation: When a local artificial stimulus is applied to any portion of the ventricular surface the impulse spreads somewhat radially from the point of stimulation and induces a series of local fractionate contractions responsible for the initial slow rise of intraventricular pressure. This continues until the impulse has reached the His-Tawara system and has been conducted by this system to the unexcited portions of the ventricle. Consequently, two different contraction processes almost imperceptibly merge, viz., *a*, a localized fractionate contraction occasioned by a relatively slow fiber to fiber excitation, and *b*, a more generalized contraction of the remaining ventricular muscle excited via bundle branches in more rapid sequence. Inasmuch as the latter also starts in a fractionate manner the isometric contraction and total systole are prolonged. While this double contraction process also contributes to the decrease in total tension developed, evidence is cited that this is further influenced by the abnormal directional spread of impulses over the conducting system. Such changes in distribution (fig. 4) may alter the order in which the muscle scrolls are excited and this in turn may affect the effectiveness of contraction of the entire ventricle as gauged by tension development. Further, opposing impulses may mutually annihilate one another and thus cause the elimination of some fractionate contractions from the entire ventricular effort.

Such pronounced effects of abnormal modes of excitation on the resulting contraction curve of the entire ventricle must be taken into account in evaluating the reactions of the ventricle to artificial stimuli.

3. Stimuli given just previous to the beginning of a normal ventricular systole always cause smaller pressure curves while those falling during the isometric contraction phase are invariably negative. Finally, stimuli occurring during the ejection phase occasionally cause larger pressure curves (fig. 6). There is no reason, however, to interpret such smaller or larger beats as evidence that the ventricle is capable of excitation or inhibition during early systole in the fundamental sense of these terms. Electrocardiographic evidence is presented that the smaller beats are either characteristic late diastolic reactions or an effect of interference of conduction waves and reasons are given why the larger beats are more probably explained by the excitation of additional fractions.

4. Stimuli applied approximately during the latter 0.06 second of systole evoke small contractions toward the end of the isometric relaxation phase. As these reactions are elicited from portions of the heart which receive their normal excitation latest, there can be no possibility of explaining these reactions as due to the excitation of fractions which have already ended their contraction period; on the contrary they indicate that the fractions are again excitable toward the end of the contraction process. This has been termed the non-refractory period of systole. No evidence was obtained that such excitations have the effect of prolonging the systole during which they fall or that they produce superimposed contractions; indeed analysis shows that such effects are physically quite impossible and dynamically would be absolutely valueless. The duration of this non-refractory phase of systole varies directly with the intensity of stimuli and with the duration of total contraction process.

5. Mammalian experiments confirm the observations previously made on amphibian hearts that the earlier the stimulus is given the longer the latent period for response. It is not necessary, however, to assume peculiar recovery gradients to explain these results; the latent interval is only an apparent one in any case and its lengthening in the case of earlier stimuli is accounted for by resultant effects of simultaneous contraction and relaxation forces.

6. As is well known, the earlier that contractions come during diastole the smaller the total energy developed. There is comparatively little evidence that the beneficial effect of a longer recovery period plays more than a very minor rôle in the case of mammalian hearts. The earlier that contractions fall in the isometric relaxation phase, the smaller the amplitude of the pressure elevation as measured by the vertical height of the ascending limb, on the other hand the maximum tension developed is progressively less as the beats come later.

The later that premature contractions begin after the A-V valves have

opened and the ventricle has begun to fill, the larger the amplitude becomes.

An analysis of results indicates that this is chiefly determined by the degree of filling and the initial stretching of the ventricular muscle.

7. A comparison of beats during the isometric relaxation and filling phase of ventricular diastole point clearly to the view that initial length of muscle fiber fundamentally determines the vigor of response. This in no way comprises the position, however, that such changes in initial length are solely produced by previous changes in initial tension when the cycle is long enough to incorporate a reasonable inflow phase.

8. Premature ventricular contractions may be *effective*, i.e., cause a systematic expulsion of blood or they may be *ineffective* as regards such ejection. The fundamental factor determining effectiveness of beats is a sufficient interval for ventricular filling under different conditions of auricular pressure. Under normal experimental conditions, a minimal filling interval of 0.04 second is required but occasionally an interval 0.055 is not sufficient. The interval is reduced when venous pressures are augmented by infusion, and slightly affected also by the diastolic pressure. The effects of the completeness of previous ventricular systole and of changes in ventricular relaxation rate are briefly discussed.

BIBLIOGRAPHY

ADRIAN, E. D. 1920. Journ. Physiol., liv, 1.

CARLSON, A. J. 1907. This Journal, xviii, 82.

CUSHNY, A. R. AND S. A. MATHEWS. 1897. Journ. Physiol., xxi, 213.

DRURY, A. N. 1923. Heart, x, 405.

GLEY, E. 1889. Arch. de physiol. normal et path., i, 499.

HERING, H. E. 1905. Verhandl. d. Kongress f. inn. Med., xxii, 205.

HIRSCHFELDER, A. D. AND J. A. E. EYSTER. 1907. This Journal, xviii, 222.

LEWIS, T. 1916. Phil. Trans. Roy. Soc., London, 207, 247.

LEWIS, T. 1920. The mechanism and graphic registration of the heart beat. London, 212.

LEWIS, T. AND M. A. ROTHSCHILD. 1915. Phil. Trans. Roy. Soc. London, 206, 181.

MACWILLIAM, J. A. 1923. Proc. of the xith Internat. Physiol. Congress., Quart. Journ. Exper. Physiol., Suppl. Volume.

RIHL, J. Zeitschr. f. exper. Path. u. Therap., 1907, iv, 257.

SCHULTZ, W. H. 1906. This Journal, xvi, 496.

TIGERSTEDT, R. 1921. Physiologie des Kreislaufes (2nd. ed.) ii, 26.

WASTL, H. 1922. Zeitschr. f. Biol., 1922, lxxv, 289.

WIGGERS, C. J. 1914. This Journal, xxxiii, 382.

1921a. This Journal, lvi, 439.

1921b. This Journal, lvi, 415.

1921c. Proc. Soc. Exper. Biol. and Med., xviii, 144.

1921d. Arch. Int. Med., xxvii, 485.

1923. Circulation in health and disease. Philadelphia, 2nd ed., 111.

WIGGERS, C. J. AND W. R. BAKER. 1924. Journ. Lab. Clin. Med., x, 54.

WIGGERS, C. J. AND H. FEIL. 1922. Heart, ix, 149.

WIGGERS, C. J. AND L. N. KATZ. 1922. This Journal, lviii, 439.

WOODWORTH, R. S. 1903. This Journal, viii, 213.

THE UTILIZATION OF MINERALS BY EWES DURING THE PERIOD OF GESTATION[1]

A. R. WINTER

From the Department of Animal Industry, Ohio Agricultural Experiment Station, Wooster, Ohio

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Present day knowledge of the utilization of minerals by sheep is very limited. Fraps (1) has probably contributed the most extensive data on the subject. While studying the digestibility of a large number of roughages he made a number of mineral balances. The results indicate that for mature sheep the daily requirements per 100 pounds live weight are: 1.7 grams phosphoric acid, 2.8 grams calcium oxide and 1 gram magnesium oxide.

The extensive mineral metabolism experiments reported from this Station have not included investigations with sheep. Since so few data are available on the utilization of minerals bysheep it was deemed advisable to extend this type of work. The experimental work to be reported here is part of a project which was originally planned for another purpose. This work was done for the purpose of 1, determining the mineral metabolism of sheep; 2, determining the change in calcium metabolism of ewes during the period of gestation.

Experimental. The general scope of the experiment is given in table 1. Five C type four-year-old ewes were used in this experiment. They were confined in metabolism crates, originally used by Forbes (2) for swine. The daily ration consisted of 700 grams roughage, 460 grams concentrate, 12 grams sodium chloride, and in periods supplemented by calcium, 16 grams precipitated bone flour. Distilled water was given ad libitum unless otherwise stated. The concentrate used throughout the experiment was prepared at one time. It consisted of 4 parts oats, 2 parts yellow corn and 1 part linseed meal. The quality of the alfalfa hay was poor. The timothy hay fed sheep 1 and 2 was rich in calcium while that fed sheep 3 and 5 was poor in calcium.

The preliminary periods, in case of a change of ration, consisted of 10 days and the collection periods as stated in table 1. The method of procedure was similar to that formulated by the National Research Council (3).

[1] Published with the permission of the Director of the Ohio Agricultural Experiment Station, Wooster, Ohio.

The analytical methods used were those reported in Ohio Agricultural Experiment Station Bulletin 363. All determinations were made in triplicate.

Discussion. The experiment extended over a period of five months, from November, 1923, to April, 1924. The sheep seemed to be especially well adapted for metabolism studies. Although confined in cages with little chance for exercise all, except sheep 5, went through the entire time

TABLE 1

General scope of experiment

SHEEP NUMBER	PERIOD NUMBER	LENGTH	PERIOD OF GESTATION	DISTINGUISHING FEATURES OF RATION
		days	*days*	
1	1	20	56–75	Timothy hay, surface water
	2	20	85–104	Timothy hay
	3	20	115–134	Timothy hay, precipitated bone flour
	4	5	144–148	Alfalfa hay
2	1	20	51–70	Timothy hay, precipitated bone flour
	2	20	80–99	Timothy hay, precipitated bone flour, surface water
	3	20	109–128	Timothy hay
	4	12	137–148	Alfalfa hay
3	1	20	53– 72	Alfalfa hay
	2	20	82–101	Alfalfa hay, 0.2 gram copper sulfate
	3	20	113–132	Timothy hay
	4	16	133–148	Timothy hay
4	1	19	45–63	Alfalfa hay
	2	20	74–93	Alfalfa hay
	3	20	94–113	Alfalfa hay
	4	20	114–133	Alfalfa hay
	5	20	134–153	Alfalfa hay
5	1	20	44–63	Alfalfa hay
	2	20	83–102	Alfalfa hay, precipitated bone flour
	3	20	103–122	Alfalfa hay, precipitated bone flour
	4	20	123–142	Alfalfa hay, precipitated bone flour
	5	7	152–158	Timothy hay

without signs of unrest or nervousness due to handling or long confinement. Sheep 1, 2, 3 and 4 had good appetites at all times and showed no signs of metabolism disturbances. Sheep 5 was nervous and did not become well adapted to the experimental procedure. She ate little feed during period III and part of period IV and was constipated part of that time. A few days after the close of the experiment she gave birth to twin lambs, one of which was dead and the other living but weak and abnormal. The other

four sheep were kept in the cages until parturition. Sheep 1 had twin lambs and the others had 1 each, all of which were of normal size and vigor.

The constipating effect of timothy hay was prevented by feeding a small amount of linseed meal. Coarse timothy hay of high calcium content,

TABLE 2

Balance of nitrogen and minerals—grams (except calcium). Period I

SHEEP NUMBER	NITROGEN	SULFUR	MAGNESIUM	PHOSPHORUS	SODIUM	POTASSIUM	CHLORINE
	Food	Food	Food	Food	Food	Food	Food
	Urine	Urine	Urine	Urine	Urine	Urine	Urine
	Feces	Feces	Feces	Feces	Feces	Feces	Feces
	Balance	Balance	Balance	Balance	Balance	Balance	Balance
	310.2	37.3	43.4	62.3	128.5	188.9	161.1
	129.0	12.1	8.2	0.5	43.1	101.3	107.8
1	133.7	21.9	33.9	60.8	73.8	84.8	47.9
	+47.5	+3.3	+1.3	+1.0	+11.6	+2.8	+5.4
	318.7	38.8	45.8	121.1	130.5	199.5	164.9
	140.2	14.5	9.9	1.2	20.1	137.6	131.3
2	136.9	20.0	32.3	113.5	89.0	51.2	36.7
	+41.6	+4.3	+3.6	+6.5	+21.4	+10.7	−3.1
	513.8	53.8	50.7	65.0	124.6	305.0	190.6
	297.7	24.8	12.5	0.5	68.0	228.0	157.3
3	137.7	23.5	33.1	60.1	41.8	63.6	35.7
	+78.4	+5.5	+5.1	+4.4	+14.8	+13.4	−2.4
	476.3	49.8	46.9	60.8	117.4	280.0	179.5
	307.0	25.5	12.5	0.4	87.4	233.9	153.7
4	141.1	20.5	17.3	59.1	24.4	35.5	18.1
	+28.2	+3.8	+17.1	+1.3	+5.6	+10.6	+7.7
	510.9	53.5	50.4	64.8	124.4	302.7	190.2
	307.5	25.4	12.1	0.6	64.6	227.9	145.5
5	129.7	22.4	34.3	61.1	41.7	49.9	30.6
	+73.7	+5.7	+4.0	+3.1	+18.1	+24.9	+14.1

which was probably grown on rich soil, appeared to be more palatable than timothy of finer texture and low calcium content, which had been grown on poor acid soil. The water consumption and volume of urine were considerably larger when alfalfa hay was fed in place of timothy. Hart (4) and Severson (5) have observed the diuretic properties of alfalfa hay on

farm animals. The hay contained a higher percentage of magnesium and potassium salts. Such salts possess diuretic properties and probably account for the difference.

TABLE 3

Calcium balances—grams

SHEEP NUMBER		PERIOD 1	PERIOD 2	PERIOD 3	PERIOD 4	PERIOD 5
		Food	Food	Food	Food	Food
		Urine	Urine	Urine	Urine	Urine
		Feces	Feces	Feces	Feces	Feces
		Balance	Balance	Balance	Balance	Balance
1		70.5	73.2	149.6	54.7	
		1.1	1.1	1.6	0.7	
		75.8	76.6	146.2	50.5	
		−6.4	−4.5	+1.8	+3.5	
2		150.2	150.6	73.6	115.8	
		8.8	3.4	1.9	1.1	
		135.8	155.5	69.0	106.2	
		+5.6	−8.3	+2.7	+8.5	
3		198.7	198.4	24.9	17.1	
		5.9	5.5	0.9	0.6	
		203.9	198.1	31.3	20.2	
		−11.1	−5.2	−7.3	−3.7	
4		181.5	196.4	199.0	199.0	199.1
		2.9	1.4	1.9	1.1	1.0
		202.5	208.1	201.2	198.2	192.1
		−23.9	−13.1	−4.1	−0.3	+6.0
5		196.9	266.0	148.5	271.7	8.8
		11.5	1.8	0.9	2.0	0.5
		202.7	261.1	159.8	271.0	12.1
		−17.3	+3.1	−12.2	−1.3	−3.8

The coefficients of digestibility of the rations were determined for period 1. The nutritive ratio of the timothy hay ration was 1:9.7 and that of the alfalfa 1:4.6.

The intake and outgo of nitrogen and minerals other than calcium are given in table 2, while all the calcium balances are presented in table 3. All sheep were in positive nitrogen balance. The sulfur balances were also positive, as might be expected, because of the large nitrogen retention.

Undoubtedly the timothy hay ration did not furnish the maximum amount of sulfur that could be utilized economically, because with a 37 per cent increase in intake the amount retained was proportionately greater. The excess of increased intake of sulfur was excreted by the urine while the amount eliminated by the feces was approximately the same regardless of ration—as in case of the nitrogen. The influence of rations rich in sulfur, upon the quantity and quality of wool produced, is a fertile field for investigation.

The potassium balances were all positive. On the alfalfa hay rations the intake of potassium was greatly increased because of the high percentage (1.94) of potassium in the alfalfa. It is very noticeable that where the potassium intake was high, there was a loss of calcium, until late in the period of gestation. According to Gross (6), potassium is antagonistic to calcium. That might be the condition here and may, in part, account for the negative calcium balances on the alfalfa hay rations. Most of the potassium excreted was by way of the urine.

The sodium balances were all positive. The intake on both rations was about the same; but on the timothy ration the most of the sodium excreted was by way of the feces while on the alfalfa ration the most was excreted by the urine. Apparently the high potassium intake influenced the absorption and excretion of sodium.

The chlorine balances were variable and showed no consistent relationship to either sodium or potassium. Much more chlorine was excreted by the urine than by the feces.

The magnesium balances were positive. The excessive intake brought about by feeding alfalfa hay was excreted by the urine. Much more magnesium than calcium is excreted by the urine. Large negative calcium balances were accompanied by the largest storage of magnesium, but there are not enough balances available to show whether there really existed any antagonistic action between calcium and magnesium.

The phosphorus balances were all positive but only slightly so. The feeding of precipitated bone flour to sheep 2 increased the storage of phosphorus and indicated the utilization of 9 per cent of the increased intake. It increased the phosphorus content of the urine slightly. The balances reported here indicate that the smaller the amount of phosphorus stored, the greater the negative calcium balance. The addition of calcium phosphate to such rations as these would most likely improve them for it might increase the storage of phosphorus and a liberal intake of this element is also conducive to better retention of calcium. As in the case of calcium, there is very little phosphorus excreted by way of the urine.

The calcium balances indicated very unexpected results. Most mineral metabolism studies with farm animals have been conducted with growing or lactating animals. As this work included only dry pregnant ewes,

it was expected that all the calcium balances would be positive. The only noticeable difference looked for, depending on the character of the ration, was a slight difference in the storage of this element. Out of twenty-two balances of calcium covering nearly all the stages of the gestation period, and consisting of a variety of sources and differences in the quantity of the element consumed, fifteen balances were negative.

Sheep 1 was in negative calcium balance on the timothy hay ration and surface water (secured from a typical source of water for animals in the average pasture field). The change from surface to distilled water made little change in the calcium balance. A comparison was made between the two kinds of water because distilled water has been used in nearly all mineral metabolism experiments. According to Hawk (7), it is not known what influence constituents in solution of ordinary water may have on metabolism. In this case, the surface water furnished only 1.9 grams of calcium during the entire period and in no case would the amount carried in solution be the important factor. The addition of precipitated bone flour changed the balance to positive with the apparent utilization of 8 per cent of the added calcium. The change to alfalfa hay in period IV resulted in the storage of nearly 1 gram of calcium per day, which was greater than that of the previous period. It is very probable that the increased storage was due to the fact that in the very last few days of gestation the mother has increased mineral metabolism powers for building and strengthening the bones of the embryo. This sheep probably had greater power for she was collecting calcium for twins.

Sheep 2 was in positive calcium balance on the timothy hay ration with the bone flour supplement. On the same ration in period II, with the change from distilled water to tap water, the balance was negative. There was a reduction in the amount excreted by way of the urine and an increase by way of the feces, indicating a change in metabolism. The negative balance probably indicated the throwing off of excessive supplies, for in the following period, a positive balance resulted on the same ration without the mineral supplement. In period IV, the results were similar to sheep 1 under similar conditions.

Sheep 3 was in negative calcium balance on the alfalfa hay ration. This was unexpected because of the high calcium intake afforded by this legume. The poor use made of calcium of alfalfa hay under similar circumstances has been observed by Meigs (8) and Gaessler (9). In period II, and on the same ration, 0.2 gram copper sulfate was fed daily with the grain. As copper sulfate is often used as a vermifuge and is a constituent of some mineral mixtures, it was desired to observe its influence on the metabolism of calcium. During the end of the period a purgative effect of the copper sulfate was noticed with increased putrefactive odors of the feces. The negative calcium balance was reduced, but this is not attributed

to the influence of copper sulfate, but rather to decreased stores of readily available calcium in the skeleton and the demand for calcium for the embryo with the advancing period of gestation. A change to timothy hay of low calcium content in period III resulted in a distaste for the ration, less consumption, and greater loss of calcium. During period IV, which extended to the time of parturition, the ewe was still losing calcium.

Sheep 4 was maintained on the alfalfa hay ration throughout the entire experiment. At first this ewe was in negative calcium balance but as time advanced the loss was gradually reduced, came to equilibrium, and storage took place during the last few days of gestation. The loss was probably decreased because of the diminished quantity of available calcium reserve in the bones, which was brought about by the greater excretion than intake covering the extended period of time. Storage took place in the last days of intra-uterine life because of the increased demand of the fetus for calcium. This is in accordance with the work of Meigs (8) and Forbes (10). According to Forbes (11), this sheep contained about 480 grams calcium and during the time of observation 35 grams of calcium in excess of intake were excreted and a lamb containing 35 grams calcium was developed. Approximately 14 per cent of the calcium of the body of this sheep was moved during the time of observation. The excretion of calcium by way of the feces alone in excess of intake has been observed by many investigators with different kinds of animals. A satisfactory explanation of the factors influencing the action of calcium under such conditions is still lacking.

Sheep 5 was in negative calcium balance on the alfalfa hay ration. The addition of precipitated bone flour changed the balance to positive. In period III the balance was negative and is attributed to metabolism disturbances which were very evident. In period IV the balance was still slightly negative. In period V on the timothy hay ration the balance was negative and compared with sheep 3 under like conditions.

Four lambs were chloroformed at birth, and analyzed for calcium and phosphorus. The smaller twin of sheep 1 contained 35 grams calcium and 24 grams phosphorus. The lamb from sheep 4 contained 27.4 grams calcium and 18.4 grams phosphorus. Two lambs taken from the station flock contained 30.4 and 31.9 grams calcium and 19.3 and 18.5 grams phosphorus respectively.

SUMMARY

Merino sheep make good experimental animals for mineral metabolism investigations.

Balanced rations of alfalfa or timothy hay and grain mixture, with 12 grams of sodium chloride daily, fed to pregnant ewes in the early period of gestation, gave positive results in all of five balances of nitrogen, sulfur, potassium, sodium, magnesium and phosphorus.

Pregnant ewes were not able to assimilate the calcium of the alfalfa hay and timothy hay used in this experiment in a satisfactory manner.

The average calcium content of four lambs at birth was 31 grams and of phosphorus 20 grams.

The mineral metabolism of sheep is similar to that of goats and cows.

BIBLIOGRAPHY

(1) FRAPS: Texas Exper. Sta., Bull. 232, 1918.
(2) FORBES, BEEGLE, FRITZ AND MENSCHING: Ohio Agric. Exper. Sta., Bull. 271, 1914.
(3) National Research Council, Bull. 6, 1923.
(4) HART AND HUMPHREY: Journ. Biol. Chem., 1914, xix, 138.
(5) SEVERSON: Penna. Agric. Exper. Sta., Report., 1915–16.
(6) GROSS AND UNDERHILL: Journ. Biol. Chem., 1922, lvi, 119.
(7) HAWK: Biochem. Bull., 1914, iii, 430.
(8) MEIGS, BLATHERWICK AND CARY: Journ. Biol. Chem., 1919, xl, 478.
(9) GAESSLER AND MCCANDLISH: Journ. Biol. Chem., 1923, lvi, 677.
(10) FORBES, SCHULZ, HUNT, WINTER AND REMLER: Journ. Biol. Chem., lii, 298.
(11) FORBES AND KEITH: Ohio Agric. Exper. Sta. Research Bull. 6, 106, 1914.

CARBOHYDRATE STUDIES

I. The Relative Sweetness of Pure Sugars

ALICE BIESTER, MILDRED WEIGLEY WOOD and CECILE STONE WAHLIN

From the Division of Home Economics, University of Minnesota[1]

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Although the consumption of sucrose has increased rapidly during the past century (Andrews, 1917), (Palmer, 1917), (U. S. Dept. of Agric., 1923) and attempts have been made to supplement the supply of this sugar with other carbohydrates, such as glucose and maltose, and although sugars are probably used as much for their sweet flavor as for their nutritive value, accurate information as to the relative sweetness of pure sugars is lacking.

Sucrose ordinarily serves as a standard for sweetness with which other sugars are compared. The numerical values assigned to glucose or to products with a high content of this carbohydrate are illustrative of the discrepancies occurring in rating sweetness. Abel (1913) states that "cane sugar is about two and one-half times as sweet as grape sugar." Corn sugar is said to possess 45 per cent of the sweetness of cane sugar, and corn syrup, 20 per cent. (Anonymous, 1918.) Ayers, Williams and Johnson (1918) suggest ice cream mixtures in which corn syrup furnishes the sweetness of 50 per cent of its weight in cane sugar, and corn sugar 80 per cent of its weight. According to Ruehe (1918), glucose has 60 per cent the sweetening power of sucrose, and corn syrup, 80 per cent. The Water and Beverage Laboratory (Sale and Skinner, 1922) rates sucrose as 100 and dextrose as 50, while Paul (1921) assigns a value of 0.26 to glucose, 0.52 to dextrose, and 1.0 to sucrose. These discrepancies may be explained possibly by differences in methods used for comparing sweetness or by the presence of compounds other than sugars which may influence the judgment of the investigators.

During the World War the desirability of having more accurate information concerning relative sweetening powers was repeatedly demon-

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strated in attempting to conserve the supply of cane and beet sugar. The experiments reported herein have to do with the determination of the relative sweetening power of xylose, rhamnose, dextrose, levulose, galactose, sucrose, maltose, lactose and raffinose. Most of the sugars chosen occur separately or in combination with other compounds in commercial sweetening agents. The rarer sugars studied—xylose, rhamnose, galactose and raffinose—have been available in small quantities only and have been used primarily for research in fields such as medicine or bacteriology. In making the above selection it seemed desirable to include sugars which had not come into wide technical usage for the rare sugars of the present may be among the common sugars of the future.

Experimental. Physiologists and psychologists quite generally recognize four fundamental taste qualities, sweet, salt, sour and bitter. The use of highly purified carbohydrates dissolved in distilled water made sweetness the outstanding quality in the solutions used. At the time that this investigation was undertaken the methods used for the establishment of numerical values for relative sweetness were not stated or if stated gave few details of procedure. In order to test the reliability of comparisons made by tasting successively sugar solutions and standard sucrose solutions, we first undertook to determine the accuracy with which experimental subjects could discriminate between the sweetness of two concentrations of the same sugar.

Accordingly a series of quantitative solutions of sucrose (Kahlbaum) and galactose (Kahlbaum) were tasted in the order of their ascending concentrations. The subject was asked to report whether the first solution tasted was "sweet" or "not sweet." Thereafter the solutions in a series were reported as "sweeter," "less sweet" or "the same" as the solution tasted immediately before the one under investigation, or the decision of "doubtful" was given. At least twenty women of good health who were mature students or members of the departmental teaching staff served as experimental subjects for each series reported. The total time required for the tasting in a single series did not exceed five minutes. Two consecutive series were never attempted, as it was thought that the element of fatigue might vitiate the results obtained. Uniform written and oral directions were given to each person at the outset. All conversation was eliminated during the experiment except in a few cases where discussion was essential for the completion of the experiment. The women participating in the investigation were requested to discuss none of the experimental details with each other.

The solutions were marked so that their concentration or chemical nature was not evident, and were placed on a table back of the experimental subject. Before starting a series and between successive tastes of solutions in a series, the experimental subject rinsed her mouth with 15 cc.

distilled water, and all visible moisture was quickly removed from the tip of her extended tongue by means of a dry absorbent cotton swab. All solutions were transferred by means of medicine droppers in uniform quantities of one drop to the tip of the extended dried tongue—a region of the tongue chosen because it has the greatest acuity for sweet sensations. All solutions were tasted at room temperature. In order to eliminate the factor of smell, the subject closed her nostrils by pressing them together with her fingers until a judgment has been reached. The decisions were recorded by symbols on appropriate blanks.

TABLE 1

Showing the accuracy with which differences in concentrations of sugar solutions may be detected by taste

SUGAR TESTED	LIMITS OF CONCENTRATIONS IN SERIES	INTERVALS IN CONCENTRATIONS IN SERIES	NUMBER OF SOLUTIONS IN SERIES	NUMBER OF EXPERIMENTAL SUBJECTS TASTING SERIES	NUMBER OF DECISIONS INDICATING SOLUTION MORE SWEET THAN PRECEDING ONE	NUMBER OF DECISIONS INDICATING SOLUTION LESS SWEET THAN PRECEDING ONE	NUMBER OF DECISIONS INDICATING SUCCESSIVE SOLUTIONS THE SAME	NUMBER OF DECISIONS INDICATING DOUBT	TOTAL NUMBER OF DECISIONS	PER CENT OF ACCURATE DECISIONS
	per cent	*per cent*								
Sucrose	0.75–5.00	0.25	18	22	223	32	97	22	374	59.6
	5.25–10.00	0.25	20	22	196	78	130	14	418	46.9
	0.75–9.75	0.50	19	21	221	20	118	19	378	58.4
	0.75–9.75	0.75	13	21	167	10	65	10	252	66.2
	0.75–9.75	1.00	10	20	135	4	38	3	180	75.0
	0.75–9.50	1.25	8	20	104	5	26	5	140	74.3
	0.75–9.75	1.50	7	21	119	0	5	2	126	94.4
Galactose	4.00–27.75	4.75	6	21	101	1	3	0	105	96.2

The arrangement of the solutions of sucrose and galactose tested was such that each solution was of a higher concentration than the preceding one, and therefore it was to be expected that the subjects might find each solution sweeter than the preceding one. An accurate decision has been defined for purposes of tabulation as one in which a solution was pronounced sweeter than the preceding solution. An examination of the data as set forth in table 1 shows that differences in concentrations are not readily detected in taste. With a series of sucrose solutions varying in concentration from 0.75 to 9.75 per cent with intervals of 0.5 per cent in the concentration, approximately three-fifths of the experimental subjects gave accurate decisions; with differences of 1.00 per cent, three-fourths

of the subjects gave accurate decisions, and with differences of 1.5 per cent, 94 per cent of the subjects gave accurate decisions. In other words, when sucrose solutions having concentrations of 0.75, 2.25, 3.75, 5.25, 6.75, 8.25 and 9.75 per cent were consecutively compared—a series representing intervals of 1.5 per cent between concentrations—the decisions were substantially accurate. In the galactose series three decisions indicated that one solution was as sweet as another having a concentration of 4.75 per cent less. One person considered such a solution of less sweetness than that of the next lower concentration. Ninety-six per cent of the total decisions were accurate. The number of inaccurate decisions made depends largely upon the sweetness of the sugar used and the size of the interval selected for the series. Of twenty subjects who participated in experimental series involving a total of 85 to 94 decisions on sucrose and galactose solutions, the lowest per cent of accurate decisions was 42.5, the highest, 91.5 and the mean, 64.0 per individual subject. The accuracy of decisions for solutions of a lesser concentration than 5.0 per cent is slightly lower than that for the parts of series above 5 per cent. Subsequent experiments with sucrose solutions indicated that approximately 30 per cent of twenty-three experimental subjects did not regard a 0.75 per cent solution of sucrose, the initial solution of the series, as sweet. This may explain the slightly larger number of inaccuracies in the judgments concerning solutions of lower concentrations.

A consideration of the above results indicates the unreliability of comparative sweetness tests in which memory plays a part. It therefore seemed more feasible to determine the lowest concentrations of xylose, rhamnose, dextrose, levulose, galactose, sucrose, lactose, maltose and raffinose which were judged sweet by the experimental subjects and to use these minimum concentrations in establishing numerical values.

Hollingworth and Poffenberger (1917) point out that the two psychological problems of greatest interest in measuring the keenness of taste are 1, the determination of the faintest stimulus which can be sensed; and 2, the change in the amount or intensity of stimulus required to perceive a change in the taste sensation. Kahlenberg (1898) determined the lowest concentrations of several acid solutions which tasted acid when the experimenter proceeded from solutions of lower concentration to those of higher concentration. Additional investigations on the relation of chemical composition and physical state of compounds to acid taste have been reported by Corin (1887), Richards (1898), Kastle (1898), Kahlenberg (1900), Crozier (1916), Gibson and Hartman (1919) and Harvey (1920).

One series of solutions for each sugar was tasted going from those of lower concentration to those of higher concentration, this order, however, being unknown to the taster. Thus the sense of fatigue and the factor

of contrast were so controlled that conditions were favorable to the detection of the sweet quality in dilute solutions. The same general precautions observed in the first series of experiments were maintained. The amounts of solutions applied were constant, the location and area on the tongue subjected to stimulation were approximately the same, the temperatures of the solutions did not vary widely, and water was the stimulus which always immediately preceded that of the sugar solution. Smell was eliminated and the tongue was dried so that the sugar solution was not diluted by distilled water and saliva. Each solution was tasted two consecutive times, so that the second decision acted as a check on the first. The experimental subjects received uniform directions and were instructed to report that a solution was "sweet," "not sweet" or "doubtful."

The sugars used were obtained from various sources: xylose (Eimer and Amend), rhamnose (Daiggle Co.), glucose (Bureau of Standards), fructose (Pfansteil), galactose (Kahlbaum), sucrose (Kahlbaum), lactose (Noyes Bros. & Cutler), maltose (manufacturer undertermined) and raffinose (Merck). The stock solution from which subsequent dilutions were made was prepared by making a known weight of carbohydrate up to known volume. The concentration of the solution was checked by using a Schmidt and Haensch saccharimeter. Wherever practicable the original stock solution was used and in those cases in which the concentrated solution was very limited in amount, one of the dilutions which was available in larger quantities was used. All checked closely with the exception of maltose in which a 1 per cent dilution gave a value of 1.09 per cent with the saccharimeter. A slight test for dextrin was obtained with this solution, but inasmuch as the optical activity for the several dextrins is markedly greater than that of maltose (Mathews, 1920), the amount of dextrins present was probably not great.

The per cent of decisions indicating the detection of sweetness for the concentrations of sugar solutions tested is given in table 2 and is shown graphically in figures 1 and 2 in which the concentrations of the solutions are plotted on the vertical axes and the per cent of decisions indicating sweetness on the horizontal axes. By continuing the curves for galactose, sucrose and lactose so that they intersect the 100 per cent axis, there is obtained the lowest concentration of each sugar which would be reported as sweet by all experimental subjects. In table 3 are given the results of these experiments expressed in three different ways—1, the concentration indicated as sweet in the decisions; 2, the numerical ratings of the sweetness of the pure sugars taking sucrose as 100; and 3, the units by weight of pure sugars equivalent to one part by weight of sucrose.

Discussion. The value of 74.3 obtained for dextrose is higher than the value of 60 given by Ruehe (1918), of 50 given by the Water and

Beverage Laboratory (Sale and Skinner, 1922), and of 52 given by Paul (1921). The rating of 173.3 for levulose is likewise higher than that of 103 given by Paul (1921). The latter assigned a value of 28 to lactose, while we have rated it as 16.0 when sucrose is given a value of 100. The method used in our investigation did not involve the memory of the sweetness of the last solution. Hollingworth and Poffenberger (1917) state that taste images are weaker, more transient and fluctuating than

TABLE 2

Showing the per cent of decisions indicating the detection of sweetness in a series of sugar solutions of ascending concentrations

CONCENTRATION OF SOLUTION GRAMS PER 100 CC. SOLUTION	PER CENT OF DECISIONS INDICATING THE DETECTION OF SWEETNESS IN								
	Xylose	Rhamnose	Dextrose	Levulose	Galatose	Sucrose	Maltose	Lactose	Raffinose
0.25				17.5		12.5			
0.50				22.5		33.3			
0 75				100.0		68.7			
1.00						81.3			
1.25			50.0			97.9			
1.50			75.0						
1.75			100.0						
2.00					10.0				
2.25	60.0								
2.50	70.0				22.5		27.5		
2.75	80.0								
3.00	80.0	55.0			57.5		35.0		
3.25	100.0								
3.50		87.5			75.0		72.5		
4.00		100.0			97.5		100.0		
4.75									45.0
5.00									57.5
5.25									60.0
5.50									75.0
5.75									100.0
6.00								30.9	
7.00								54.7	
8.00								97.6	

are images from other senses. Moreover, the method developed does not necessitate tasting solutions of marked sweetness in succession, thus reducing the factor of fatigue to a minimum. The method outlined for establishing numerical values for the sweetening powers of sugars is obviously not applicable to the comparison of solutions of higher concentrations than those used. The first series of experiments with sucrose and galactose shows that a difference of 1.5 per cent in sucrose solutions is detected by 94 per cent of the subjects and a difference of 4.75 per cent

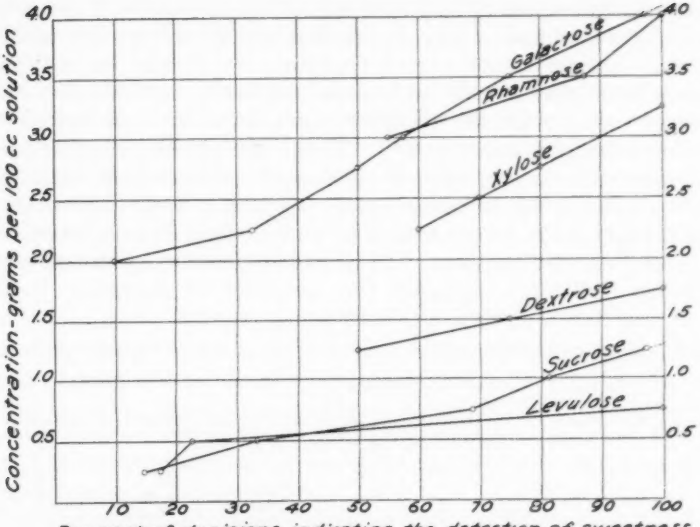
Concentration-grams per 100 cc solution
Galactose
Rhamnose
Xylose
Dextrose
Sucrose
Levulose
Per cent of decisions indicating the detection of sweetness

Fig.1

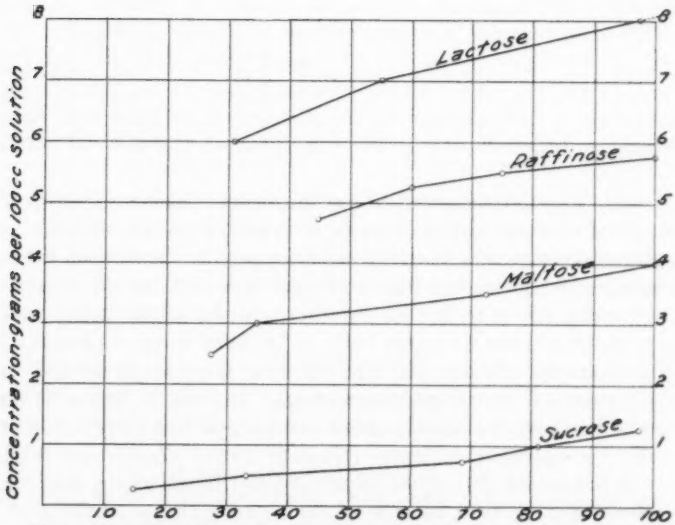
Concentration-grams per 100cc solution
Lactose
Raffinose
Maltose
Sucrose
Per cent of decisions indicating the detection of sweetness

Fig.2

in the galactose solutions by 96 per cent of the subjects; 97.9 per cent of the subjects considered a 1.25 per cent solution of sucrose sweet and 97.5 per cent consider a 4.00 per cent solution of galactose sweet. There apparently is some correlation between the lowest concentration which is sensed as sweet and the amount or intensity of a stimulus needed to perceive a change in sweet quality. Thus one might anticipate a numerical relationship among solutions of higher concentrations than those tested, similar to that obtaining among solutions of lower concentrations.

Of the sugars which are available in the pure form or as important constituents of sweetening agents, sucrose has the greatest sweetening power. Willaman (1921) has suggested the possibility of producing levulose syrup from the Jerusalem artichoke which contains 12 to 14 per cent inulin. Such a product would undoubtedly be much sweeter than other

TABLE 3

Showing the concentration indicated as sweet by all experimental subjects and the numerical ratings of the several sugars

SUGAR	CONCENTRATION REPORTED SWEET BY ALL SUBJECTS	NUMERICAL RATING (SUCROSE = 100)	UNITS OF WEIGHT OF SUGAR EQUIVALENT TO ONE UNIT OF SUCROSE
Lactose	8.10	16.0	6.3
Raffinose	5.75	22.6	4.4
Galactose	4.05	32.1	3.1
Rhamnose	4.00	32.5	3.1
Maltose	4.00	32.5(?)	3.1(?)
Xylose	3.25	40.0	2.5
Dextrose	1.75	74.3	1.3
Sucrose	1.30	100.0	1.0
Levulose	0.75	173.3	0.6

syrups with a similar per cent of carbohydrate. Levulose syrup would be a good sweetening agent to be used with carbohydrates of lesser sweetening value or for cases in which a sweet product is desired without a correspondingly high carbohydrate content and high caloric value. The low sweetening power of lactose together with its high caloric value has brought about its use in fevers such as typhoid fever (Coleman, 1912) where commercial glucose and sucrose soon become distasteful to the patient because of their greater sweetness. The use of lactose is limited on the one hand by the carbohydrate tolerance of the patient and on the other, by its solubility in water (Hudson, 1908), (Zoller and Williams, 1921). It is possible that other sugars studied in the group may come to have special dietetic uses because of their sweetening qualities and their physiological behavior. Raffinose is not utilized by the human organism (Kuriyama and Mendel, 1917), (Kuriyama, 1918). Investigations re-

ported on the pentoses indicate that they do not behave like the hexoses in the animal body (Armsby, 1908), (Lusk, 1917), (Rockwood and Khorizian, 1921).

Theoretically the hydrolysis of raffinose, lactose, maltose and sucrose would result in a mixture of monosaccharides having greater sweetening power than the original sugar, provided that the process chosen does not modify the simple sugars produced. The only one of these mixtures which would be sweeter than sucrose would be that derived from sucrose, itself, namely, invert sugar. The sweetness of the latter will be considered in a subsequent communication.

SUMMARY

When solutions of sucrose and galactose of ascending concentrations are compared with a solution of lower concentration in a series, different degrees of sweetness are not accurately detected by taste. The difference in concentration must be 1.5 per cent in the case of sucrose and 4.75 per cent in the case of galactose before the difference in sweetness is detected with reasonable accuracy.

Therefore a method of comparing the sweetness of sugars has been developed which does not involve remembering a sweet sensation from one test to the next. By this method the minimum concentration of each sugar which is declared sweet by at least 20 experimental subjects has been established. Using the value of 100 for sucrose, the relative sweetness of lactose, raffinose, galactose, rhamnose, maltose, xylose, dextrose, sucrose and levulose has been determined.

Theoretically the monosaccharides obtained from the complete hydrolysis of raffinose, maltose, lactose and sucrose would have a greater sweetening power than a corresponding amount of unhydrolyzed carbohydrate.

The writers are indebted to Prof. J. J. Willaman and Prof. C. A. Morrow of the Division of Agricultural Biochemistry for many helpful suggestions and criticisms. They gratefully acknowledge the coöperation and assistance rendered by the group of women who served as experimental subjects in this investigation.

BIBLIOGRAPHY

ABEL, M. H. 1913. U. S. Dept. Agric. Far. Bull. 535.
ANDREWS, F. 1917. U. S. Dept. Agric. Yearbook Separate 756.
ANONYMOUS. 1918. Amer. Food Journ., xiii, 384.
ARMSBY, H. P. 1908. Principles of animal nutrition. Ed. 3.
AYERS, S. H., O. E. WILLIAMS AND W. T. JOHNSON. 1918. Creamery and Milk Plant Monthly, vii, 48.
COLEMAN, W. 1912. Amer. Journ. Med. Sci., cxliii, 77.
CORIN, J. 1887. Arch. de Biol., viii, 121.

CROZIER, W. J. 1916. Journ. Comp. Neurol., xxvi, 453.
GIBSON, L. AND T. HARTMAN. 1919. This Journal, xxx, 311.
HARVEY, R. B. 1920. Journ. Amer. Chem. Soc., xlii, 712.
HOLLINGWORTH, H. E. AND A. T. POFFENBERGER. 1917. The sense of taste.
HUDSON, C. S. 1908. Journ. Amer. Chem. Soc., xxx, 1767.
KAHLENBERG, L. 1898. Univ. Wis. Sci. Series II.
KAHLENBERG, L. 1900. Journ. Physical Chem., iv, 533.
KASTLE, J. H. 1898. Amer. Chem. Journ., xx, 466.
KURIYAMA, S. 1918. Journ. Biol. Chem., xxxiv, 321.
KURIYAMA, S. AND L. B. MENDEL. 1917. Journ. Biol. Chem., xxxi, 125.
LUSK, G. 1917. Science of nutrition. Ed. 3, 488.
MATHEWS, A. P. 1920. Physiological chemistry. Ed. 3.
PALMER, T. G. 1917. Amer. Food Journ., xxii, 260.
PAUL, T. 1921. Chem. Ztg., xlv, 705.
RICHARDS, T. W. 1898. Amer. Chem. Journ., xx, 121.
ROCKWOOD, E. W. AND K. G. KHORIZIAN. 1921. Journ. Biol. Chem., xlvi, 553.
RUEHE, H. A. 1918. Ill. Agric. Expt. Sta. Circ. 219.
SALE, J. W. AND W. W. SKINNER. 1922. Journ. Ind. Eng. Chem., xiv, 522.
U. S. Dept. Agric. 1923. Yearbook Separate 893, 1.
WILLAMAN, J. J. 1921. Science, lii, 351.
ZOLLER, H. F. AND O. E. WILLIAMS. 1921. Journ. Agric. Res., xxi, 791.

CARBOHYDRATE STUDIES

II. The Relative Sweetness of Invert Sugar

J. J. WILLAMAN, CECILE STONE WAHLIN AND ALICE BIESTER

From the Division of Agricultural Biochemistry and the Division of Home Economics University of Minnesota

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The effect of hydrolysis upon the sweetening capacity of a given quantity of sucrose is not a new problem as is evidenced by various estimates and investigations reported in which the effect of inversion has been evaluated. Allen (1909) describes invert sugar as "an uncrystallizable syrup having a sweeter taste than cane sugar." Snow (1909), in a series of comparative tests with sugar solutions, concluded that a solution containing equal amounts of dextrose and levulose was less sweet than one with a corresponding amount of sucrose. These results were confirmed by Day (1910). Hunt and Atwater (1915) suggest that it is safe to estimate that one cup of honey which contains 9 or 10 ounces of solids, most of which are invert sugar, will sweeten a food about as much as a cup of cane sugar. Ruehe (1918), (1919) and Frandsen, Rovner and Luithley (1918) estimate that the inversion of cane sugar will increase its sweetening power 20 or 25 per cent and 22 per cent respectively. Willaman (1918) predicted that the inversion of 100 pounds of cane sugar which results in 105 pounds of invert sugar, would result in a mixture having the same sweetening power as 135 pounds of cane sugar. Sale and Skinner (1922) conclude from a series of comparative tests that 117 units of invert sugar are necessary to equal 100 units of cane sugar or that invert sugar is 85 per cent as sweet as cane sugar. The results obtained undoubtedly are quite largely explained by the methods of testing sweetness employed. Since the procedure described by Biester, Wood and Wahlin (1925) had proven satisfactory in the evaluation of pure sugars, the present writers thought it would be of interest to apply this method in the determination of the relative sweetness of invert sugar and sucrose.

Experimental. In the investigation reported herein, the sweetness of invert sugar prepared 1, by the use of invertase; and 2, by the solution of equal weights of dextrose (Bureau of Standards) and levulose (Pfansteil) has been determined. The inversion of sucrose by enzyme action involved a preliminary preparation of invertase according to the method outlined

by Hudson (1914). Dilutions were made from the two invert sugar solutions, varying by the equivalent of 0.25 per cent of sucrose in concentration. The results of the tasting experiments, in which twenty healthy women participated are summarized in table 1.

These data are graphically shown in figure 1. The curves for invertase invert sugar and for sucrose have been extended to show the concentration at which sweetness would be detected by all experimental subjects. Assigning to sucrose a value of 100, invertase sugar has a numerical value of 127.4 and synthetic invert sugar, a value of 130.0. From the factors of 74.3 for dextrose and 173.3 for levulose reported by Biester, Wood and Wahlin (1925), the invert sugar factor expected would be 130.0 when allowance is made for the increased weight of sugars resulting from inversion. According to the above values, 0.78 unit by weight of invertase invert sugar or 0.77 parts of synthetic invert sugar would be equivalent to one unit by weight of sucrose.

TABLE 1

Showing the per cent of decisions indicating the detection of sweetness in a series of invert sugar solutions of ascending concentrations

CONCENTRATION OF SOLUTIONS EXPRESSED IN EQUIVALENT GRAMS OF SUCROSE PER 100 CC.	PER CENT OF DECISIONS INDICATING DETECTION OF SWEETNESS		
	Invert sugar (synthetic)	Invert sugar (invertase)	Sucrose (Biester, Wood and Wahlin, 1925)
	per cent	*per cent*	*per cent*
0.50	42.5	32.5	33.3
0.75	62.5	45.0	68.7
1.00	100.0	97.5	81.3
1.25			97.9

Discussion. The numerical results obtained for mixtures of dextrose and levulose prepared by two methods on inversion, 127.4 and 130.0, check closely with the value of 130 which may be predicted from the values of 173.3 for levulose and 74.3 for dextrose given by Biester, Wood and Wahlin (1925). They also parallel the value of 122 suggested by Frandsen, Rovner and Luithley (1918), that of 120 to 125 reported by Ruehe (1918), (1919) and that of 135 predicted by Willaman (1918). These results do not confirm the conclusions reached by Snow (1909), Day (1910) and Sale and Skinner (1922), all of whom considered invert sugar less sweet than sucrose.

Unquestionably the cause of the above discrepancies lies in the methods employed in measuring sweetness. The greatest difficulty experienced in comparing a series of solutions is in retaining accurate memories of the relative sweetness of two or more solutions until a decision has been reached. Experiments reported by Biester, Wood and Wahlin (1925) indicated that, with intervals of 0.25 per cent, only 52.9 per cent of approxi-

mately 20 experimental subjects detected differences in sweetness accurately. Decreasing the number of solutions and increasing the intervals to 0.50 per cent gave 58.4 per cent of subjects who accurately detected differences in sweetness. Sucrose solutions were evaluated with an accuracy of 94 per cent only when the increments of concentration were increased to 1.5 per cent. It is therefore evident that an increase of 25 per cent in sweetness due to the inversion of sucrose may not be detected, if the method used necessitates the direct comparison of solutions. Since the procedure followed in the present investigation involved the comparison of the lowest concentrations in three series of sugar solutions at which sweetness could be detected by the experimental subjects, the factor of memory has been eliminated.

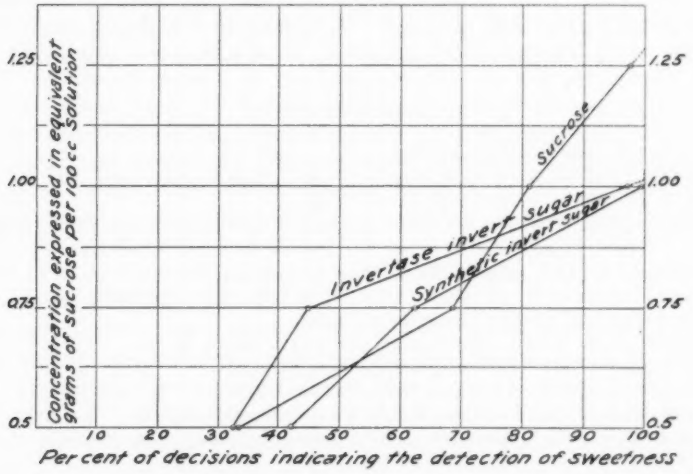

Fig. 1

Furthermore, the presence of compounds other than sugars may possibly influence decisions concerning relative sweetness. Thus Sale and Skinner (1922), who obtained lower values, report characteristic flavors associated with invert syrups prepared by the use of invertase which were not detected by the writers in the solutions which they used. Since our value (127.4) for invertase invert sugar is only 2 per cent lower than the theoretical value of 130.0, it would appear that the enzyme preparations used did not markedly affect the results. Lord (1917) and Ogilvie (1922) report that salts apparently increase the sweetening power of sugars. The latter cites experiments reported by Zuntz indicating that bitter compounds associated with sugar also tend to increase the sweetness of the resulting

mixture. The monosaccharides are known to decompose slowly in acid solution (Mathews, 1920). This may prove to be a significant factor in lowering the sweetness of sugars inverted by acids.

SUMMARY

The concentrations of invert sugar prepared 1, by the use of invertase, and 2, by the solution of equal weights of dextrose and levulose, at which twenty subjects detect sweetness, have been determined and compared with similar data for sucrose. Taking into account the fact that 100 parts of sucrose yield 105 parts of dextrose and levulose by weight and assigning to sucrose the value of 100, the numerical value for invertase (invert) sugar is 127.4 and that for synthetic invert sugar is 130.0. Thus the inversion of sucrose by either of the methods described increases its sweetening power from 27.4 to 30 per cent. The effect of compounds other than sugars, upon relative sweetness has not been included in this study.

BIBLIOGRAPHY

ALLEN'S Commercial organic analysis, i, ed. 4, 1909.
BIESTER, A., M. W. WOOD, C. S. WAHLIN. 1925. This Journal, lxxiii, 387.
DAY, E. D. 1910. Journ. Home Econ., ii, 94.
FRANDSEN, J. H., J. W. ROVNER AND J. LUITHLY. 1918. Neb. Agric. Expt. Sta. Bull. 168.
HUDSON, C. S. 1914. Journ. Amer. Chem. Soc., xxxvi, 1566.
HUNT, C. L. AND H. W. ATWATER. 1915. U. S. Dept. Agric. Far. Bull. 653.
LORD, I. E. 1917. Journ. Home Econ., ix, 375.
MATHEWS, A. P. 1921. Physiological chemistry. Ed. 3, 35.
OGILVIE, J. F. 1922. Internat. Sugar Journ., xxiv, 288.
RUEHE, H. A. 1918. Ill. Agric. Expt. Sta. Circ. 219.
RUEHE, H. A. 1919. Creamery and Milk Plant Monthly, viii, 45.
SALE, J. W., AND W. W. SKINNER. 1922. Journ. Ind. Eng. Chem., xiv, 522.
SNOW, J. H. 1909. Journ. Home Econ., i, 261.
WILLAMAN, J. J. 1918. Amer. Food Journ., xiii, 636

THE NATURE OF THE SURFACE OF THE RED BLOOD CELL AND MECHANISMS OF SUSPENSION STABILITY

JEAN OLIVER AND LEONARD BARNARD

From the Department of Pathology, Stanford University Medical School, San Francisco, California

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Some experiments on the electrokinetic properties of red blood cell suspensions (Oliver and Barnard, 1924–25) have suggested that evidence concerning the nature of the cell surface may be obtained from such a method of investigation. It is at the interface of cell protoplasm and suspending fluid that the electric charges are concentrated and the effect of these charges should therefore vary with the condition of the interface. Any additional information that can be so obtained may be of help in explaining certain points which are still obscure.

Two questions which have arisen in our work are first, the chemical nature of the cell surface, and second, the cause of a certain type of stability which is observed in suspensions of red blood cells when their electrolyte concentration is high.

In regard to the first of these problems, a vast amount of evidence from other sources has led to the concept of the "surface membrane," and certain experiments have suggested that lipoids play an important part in the formation of this structure. Some investigators, however, Robertson (1908), for example, have shown that in many ways the surface of the cell reacts as if it were composed of proteins. Further evidence from a new viewpoint will therefore be of interest.

The second problem may well be connected with the first. It has long been known that colloids may be divided into two groups, and one of the most striking differences between the two types is the difference in the mechanism by which stability of the suspension is produced. With the suspensoid or hydrophobe colloids stability is a function of the electric charge of its particles, while the emulsoids or hydrophile are not dependent on the charge of their particles for their stability. It is further known that both chemical and physical differences in the dispersed particles may be responsible for the type of reaction, whether suspensoid or emulsoid, of the solution.

In the case of suspensions of red blood cells Northrup and Freund (1923–24) and Oliver and Barnard (1924–25) have shown independently that the

charge of the suspended cells is of importance as a stabilizing factor, *except* when there is a high concentration of electrolyte in the suspending fluid. Under these conditions there is no apparent causal relation between the electric charge of the cells and the stability of the suspension. Northrup and Freund have suggested an explanation for this apparent anomaly which we will discuss later in this paper.

It is our purpose now to investigate more closely these two phases of the problem, the nature of the cell surface and the hydrophilic type of stability observed in high electrolyte concentration, and if possible to correlate the experimental findings.

METHODS. Rabbit red blood cells obtained from defibrinated blood were used in all the experiments. A 5 per cent stock suspension of them as free as possible of electrolyte was prepared by washing the cells repeatedly in isotonic sucrose solution until the supernatant fluid gave no reaction for chlorides with $AgNO_3$. To portions of this, a solution of electrolyte was added so as to form a series of electrolyte concentrations.

The stability of the suspension was observed in test tubes which were allowed to stand for one hour at room temperature. This time was chosen because differences in the variations in the stability of the suspension which develop as an effect of the experimental procedure are often overlooked if the observation is made at a later time. Moreover chemical reactions occur with many of the electrolytes which after a certain length of time complicate the experiment to such a degree that the immediate physical reactions, in which alone we are interested, are completely obscured. After several hours with Al_2Cl_6 in high concentration for example, the cells are coagulated in a firm mass and the hemoglobin turned a dark brown.

The degree of instability of the suspension or agglutination of the cells is expressed as + when the supernatant fluid was completely clear of clumped cells, as ± when a few cells remained unagglutinated and suspended in the supernatant, and as 0 when no gross agglutination of the cells could be seen.

The electric charge of the cells was determined in the usual manner by a Michaelis cataphoresis chamber and the results expressed in millivolts. The general details of this method are given in our earlier paper. In certain experiments in which the particles were too small to be directly observed by the microscope, such as is the case with an albumin solution, the cataphoretic determination was done in a U tube. The method of Burton (1921) was followed and the usual corrections made. In the charts which show the P.D. of the particle and liquid, values less than 10 millivolts are plotted as zero, as with such small charges the movement in the electrical field was so slow that accurate readings could not be made.

DOES THE SURFACE OF THE SUSPENDED CELL REACT LIKE A SUSPENSOID OR LIKE AN EMULSOID? This question may be answered by a determina-

tion of the cause of stability of the suspension. As has been stated, with suspensoids the determining factor is the P.D. at the surface of the particle, while with emulsoids other factors are concerned.

The work of Northrup and Freund and our experiments have indicated that in moderate concentrations of electrolytes the stability of the suspension depends on the electric charges of the cells. For the sake of demonstration certain of these experiments have been repeated. As a suspensoid, collodion particles in water and for an emulsoid, gelatine coated collodion particles in water were used and these contrasted to a suspension of red cells. To a series of tubes of each of these, increasing concentrations of $CuCl_2$ were added, the stability of the suspensions noted and the charge of its particles determined.

A comparison of the three curves (fig. 1) shows that the cell suspension resembles the collodion particle suspensoid in that its stability depends on the charge of its particles except in the stronger concentrations of the electrolyte, $CuCl_2$. There is however this difference, that the sign of the charge of the cells is reversed at a certain concentration of $CuCl_2$, M/4096, and in this regard there is a resemblance to the reaction noted with the emulsoid gelatin suspension.

The generally accepted theory of the physical nature of protoplasm conceives it as a semi-fluid mixture of proteins and lipoids and such a mixture has, in general, the properties of an emulsoid. This experiment is of interest therefore in demonstrating that the surface of the cell differs in certain of its properties from the interior. This difference is due to the "cell membrane" whose presence has been demonstrated by other lines of investigation.

WHAT IS THE CHEMICAL NATURE OF THE CELL MEMBRANE? The experiment described above suggests a method for further analysis of the nature of the cell membrane. Since the chemical constitution of the cell as a whole is known, it would seem that it might be possible to examine the electrokinetic reactions of its constituent substances and determine in that way which of them, if existing in a surface membrane, might be responsible for its characteristic properties. This possibility is illustrated in the previous experiment, where chemical differences between the collodion particles and the gelatin particles resulted in a reversal of the sign of the charge of the latter, as the H ion concentration of the suspending fluid reached a certain point from the hydrolysis of the $CuCl_2$.

There is considerable evidence from other sources that the cell membrane is composed of lipoids, such as cholesterol and lecithin and the other possible constituent is protein. Suspensions of these substances were therefore prepared, added to increasing concentrations of $CuCl_2$ and the charge and stability of the suspension determined.

The suspension of cholesterol (fig. 2) reacts like that of the collodion par-

ticles in that it is a suspensoid and is unstable below a certain critical potential. It differs however from the suspension of red cells in that the charge of its particles is not reversed by $CuCl_2$. The presence of this substance in the cell membrane will therefore not explain the characteristic properties of the latter.

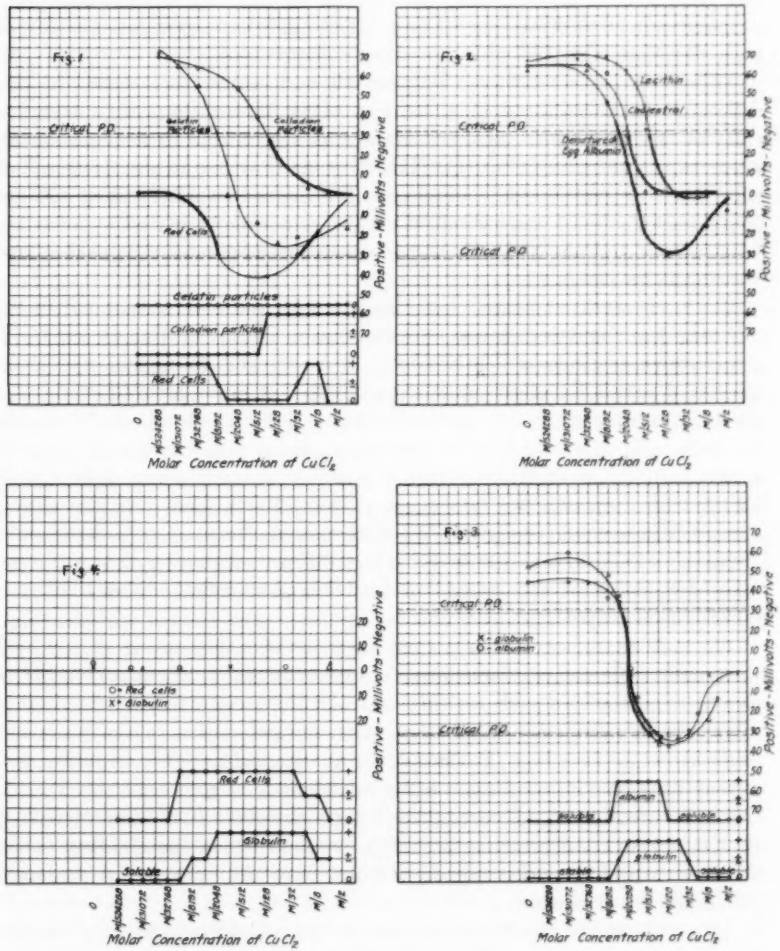
"Lecithin" (Pfanstiehl) (fig. 2) showed much the same type of reaction as did the cholesterol. The charge of its particles was depressed to an isoelectric point but not reversed and when the P.D. was below the critical potential, flocculation occurred. Lecithin was also prepared from red cells by extraction with absolute alcohol and repeated precipitation with acetone. With this preparation a slight reversal was obtained in the highest concentrations of $CuCl_2$, but examination showed that this was doubtless due to a contamination with protein, as the preparation, in contrast to the Pfanstiehl product, gave a distinct biuret and xanthoproteic reaction.

The failure of the lipoids, cholesterol and lecithin, to explain the properties of the cell membrane leads to a consideration of the proteins.

As a preliminary experiment, a suspension of egg albumin was prepared. Since it is necessary to have suspended discrete particles for the determination of the charge by Michaelis' method and since the egg albumin is soluble, it was first denatured by boiling. To this suspension $CuCl_2$ was added in the usual way and the stability and charges determined. Figure 2 shows the results. The stability of the suspension resembles that of the red cells in that flocculation occurs when the charge is below the critical potential. But the resemblance is even more perfect than has yet been found with the other prepared suspensions as there is also a reversal of the charge so that the curve of P.D. closely resembles that obtained for red cells. Except for differences in absolute values the effect on the charges is identical. There is however a difference in the stability in the higher concentrations of $CuCl_2$, for it will be noted that the denatured protein is not stable in this zone while the red cell suspension is.

Protein fractions of globulin and albumin were now prepared from plasma by precipitation with $(NH_4)_2SO_4$, dialysed free of salts, and suspended in distilled water. The albumin fraction went into solution while the globulins gave a cloudy suspension. To both were added increasing concentrations of $CuCl_2$. Figure 3 shows the resulting changes in charge and stability. The blood albumin differs in no essential respect from the denatured egg albumin except that there is a zone of stability in the high concentration of electrolytes. The behavior of suspended globulin particles is of especial interest as we see that there is a complete analogy with the red cell suspensions (figs. 1 and 3). In low and moderate concentrations of electrolyte the stability of the suspension depends on the charge alone. As the concentration of the electrolyte increases this charge

is reversed and the suspension is unstable. When the positive charge then reaches a sufficient degree, stability again occurs but as the charge is again depressed stability is nevertheless maintained in spite of the absence of electric potential.



The red cell suspended in a fluid therefore reacts in as far as its electric properties are concerned as if it possessed a surface membrane of globulin. A lipoid membrane could not account for the properties observed.

Our finding that in moderate concentrations of electrolyte the cell

surface gives the reaction of a suspensoid since its stability depends on its electric charge is apparently in disagreement with the work of Mines (1912) who came to the conclusion that a red cell suspension reacts like an emulsoid. There is an important difference in the two sets of experiments, however, as his cells were suspended in 0.9 per cent NaCl while ours were suspended in electrolyte-free sucrose. The following experiments of Mines were therefore repeated with our sucrose suspensions.

Mines' distinction between suspensoids and emulsoids is based on the difference in flocculation of these two types of solutions with complex salts of trivalent ions. Suspensoids are as susceptible to flocculation by these salts, for example by ($Co(NH_3)_6Cl_3$), as they are to simple trivalent salts such as Al_2Cl_6, while emulsoids are much less susceptible. Mines found that his NaCl suspensions of cells were not agglutinated by a certain concentration of $Co(NH_3)_6Cl_3$ and he therefore concludes that such a suspension is an emulsoid.

We have repeated Mines' experiments with red cells suspended in 0.9 per cent NaCl and find that such a suspension does react like an emulsoid. High concentrations of $Co(NH_3)_6Cl_3$, are required to agglutinate them than of a simple trivalent salt such as Al_2Cl_6. A more detailed study of a series of preparations of various substances in which the P.D. of the particles and the stability of the suspensions were determined in all concentrations of electrolyte shows however a much more complicated relationship between the two types of colloid solutions than the simple rule of Mines would suggest. We shall not at the present time describe these experiments, but simply state that Mines' contention that a 0.9 per cent suspension of red cells acts with complex cobalts salts like most emulsoids is correct.

We may therefore conclude that behavior of a suspension of red cells in regard to electrolyte flocculation, whether it reacts like a suspensoid or like an emulsoid, depends on the electrolyte concentration of the fluid in which it is suspended. When this is low, the suspension reacts like a suspensoid; when it is high, it reacts like an emulsoid. This variation also is seen of course in our former experiments with sucrose cells (fig. 1). As the concentration of added agglutinating electrolyte ($CuCl_2$) is increased, an electrolyte concentration (M/32) is reached where the stability of the suspension no longer depends on the charge of the cells, i.e., it no longer reacts like a suspensoid.

The addition of increasing concentrations of electrolyte to a sucrose suspension of red cells produces therefore two effects. As the concentration is gradually increased the first effect is on the electric charges of the cells. The second effect, reached only in higher concentrations, is to change the nature of the suspension so that it now possesses a stability of the emulsoid type. We wish now to consider the cause of this second effect.

The same problem has been studied by Northrup and DeKruif (1921–22) with bacterial suspensions. They observed that in high concentrations of electrolyte the bacterial suspension was stabile even in an absence of electric charge. This they consider due to a depression of the attractive forces (cohesion) between the cells, and an experimental determination of the adhesiveness between bacteria in the presence of electrolytes showed that *this* force was lowered as the electrolyte concentration of the surrounding fluid increased. In a later article on the agglutination of red cell suspensions, they assume that the same mechanism operates with this suspension.

The following experiment casts, however, some doubt on the applicability of this hypothesis to the red cell suspension. A suspension of red cells in strong electrolyte solution (M/4 NaCl) is quite stable, although its cells possess little or no electric charge. In such a suspension Northrup and DeKruiff assume that the cohesion between the cells is abolished and that they therefore do not agglutinate. Yet when increasing concentrations of $CuCl_2$ are added to a series of tubes of it, marked agglutination occurs in certain concentrations (fig. 4). In this zone there is no change in the P.D. of the cells which has been practically abolished in all the tubes by the strong NaCl solution. It is of particular interest that the clumps of agglutinated cells are exceedingly sticky, and can hardly be broken apart by any amount of mechanical manipulation.

One must either admit that in this experiment cohesion was not abolished by the M/4 NaCl, and the stickiness of the clumps suggests that it was not, and that some other factor was the cause of the stability of the original NaCl suspension, or else assume that the cohesion was reëstablished by the later addition of the $CuCl_2$. But it has been shown experimentally that the addition of such an electrolyte depresses the cohesion.

This dilemma is avoided if one considers our conclusion that the surface of the red cell is composed of globulin in the light of the solubility theory of colloid stability. It has been pointed out by many investigators that the stability of colloidal suspensions may be determined by other forces than differences in potential between the dispersed particle and the suspending fluid. Kruyt and de Jong (1922) have suggested that in an emulsoid, two stability factors are present, electrical charge and hydration of the particle. Thomas (1923) also emphasizes the importance of attractive forces between the particle and the surrounding medium in his "solubility theory" of colloidal dispersion. As examples of the relative importance of stabilizing forces he cites the case of oil emulsions in which the attraction between particle and water is so slight that a P.D. of 30 millivolts is required for stability (Powis); that of certain protein solutions where the attraction to water is greater and only a few millivolts are required and finally sugar solutions where the attraction between water and sugar is so

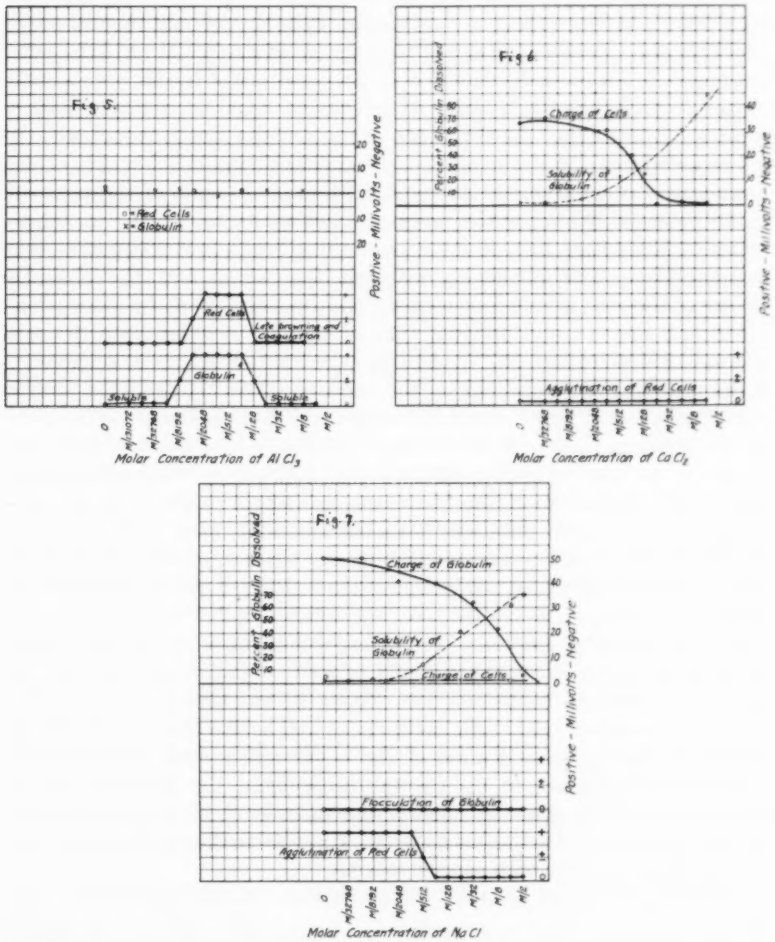
great as to be sufficient in itself for stability and no potential is required to maintain dispersion. Loeb (1924) uses the same theory to explain the stability of gelatin suspensions in the absence of electric charges, contrasting the soluble gelatin particles with insoluble collodion particles which do require a considerable P.D. to maintain stability. Chick (1913) also contrasts the "electrical type" of dispersion with the "molecular type" of solution as dispersing forces in globulin suspensions.

We have pointed out above the similarities that a suspension of red cells bears to various types of colloid dispersions and shown that it reacts in the same manner, both in regard to its electric charges and to the stability of its suspensions, as does a suspension of globulin. Now there is little doubt that the dispersion of the globulin in the higher concentrations of the experiment shown in figure 3 is due to its solubility in the salt solution. The thick turbidity of the suspension of globulin noted in the tubes of weaker concentrations of $CuCl_2$ disappears, and the preparation is clear, or slightly opalescent, in the high concentrations. The experiment with red cells suspended in M/4 NaCl may be repeated with globulin as the suspended substance. In this concentration of salt the globulin is highly dispersed in solution and quite stable and in this way resembles the stabile suspension of red cells. Figure 4 shows the stability and the charge of the globulin as increasing concentrations of $CuCl_2$ are added. As was the case with the red cells the P.D. of the globulin is practically abolished in all the tubes of the strong M/4 NaCl. Nevertheless the preparation is stable until a concentration of M/8000 is reached. A zone of flocculation follows and in the two strongest concentrations, M/8 and M/4 stability occurs again. Observation of these stable tubes shows that the suspended globulin has gone into solution, as the preparations are much clearer than a control tube in which the globulin was suspended in distilled water.

The zone of insolubility of the globulin corresponds therefore quantitatively with the zone of agglutination of the red cells. Accepting the idea that the surface of the red cell is composed in part of globulin, we see that its stability in the absence of a P.D. can be explained by the solubility forces which act in certain concentrations of electrolyte on the globulin. In that zone of concentration where globulin is insoluble, M/8000 to M/32, figure 4, this factor no longer acts, and as the cells possess no electric charge, agglutination of them occurs.

Stability of the red cell suspension may therefore be conceived as due to two factors, the P.D. of the cell as a whole and the solubility of its globulin surface. In strong electrolyte concentrations the globulin of the cell membrane is soluble, in weak concentrations it is insoluble but the cell is highly charged (fig. 3). At both these extremes of electrolyte concentration, therefore, stabilizing forces of different natures are at a maximum, while between these two zones neither stabilizing force is present, and there agglutination occurs.

This hypothesis may be tested with other combinations of electrolyte, determining and correlating in each case the stability and charge of the cell suspension with the solubility and charge of globulin. The charge of the soluble preparations of globulin was determined by the U tube



method. The solubility of it was estimated by filtering the mixtures of electrolyte and globulin and determining the protein content of the clear supernatant by the method of Shevky and Stafford (1923).

In figure 5 is shown the result obtained with increasing concentrations

of Al_2Cl_6 when added to a suspension of cells or globulin in M/4 NaCl. The findings are similar in type to those obtained with bivalent $CuCl_2$ and there is almost an exact agreement between the reaction of the red cells and globulin.

In figure 6 an experiment of a different type is represented. It shows the relations between the two stabilizing forces, P.D. at cell interface and solubility of its globulin surface, as they obtain in a series of $CaCl_2$ concentrations. To a sucrose suspension of cells a few drops of NaOH were added, enough to give the cells a high and permanent negative charge. Such a preparation is stable. As the concentration of the $CaCl_2$ is increased it will be noted that the charge of the cells decreases, passing below the critical flocculation potential of 32 millivolts at M/512. In spite of the removal of the potential difference the suspension remains stable, however, for as shown by the dotted line, 40 per cent of the globulin is in solution at this concentration. The attraction between the water and the globulin surface of the cells is sufficient to maintain the stability of the suspension.

Figure 7 shows a still different type of stability reaction with the salt of a monovalent cation, NaCl. In this experiment the sucrose suspension of red cells was not stabilized by the addition of NaOH as in the former case. As we have shown the electric charge of the cells in such a preparaton is a transient one. At first high, it gradually falls as the suspension is allowed to stand and as a result of this fall the red cells agglutinate. This is the mechanism of the "spontaneous" agglutination of sugar suspensions of red cells that has been described by many investigators.[1] It may be prevented by the addition of any electrolyte which will maintain the original high negative charge of the cells, such as NaOH or sodium citrate.

In the present experiment increasing concentrations of NaCl were added to a sucrose cell suspension and to a suspension of globulin in electrolyte free sucrose solution. The charge of globulin was determined by the microscopic and U tube methods as well as the amount in solution at each increasing concentration of NaCl. It will be seen that the P.D. is gradually depressed to 0. On the other hand the solubility in the salt gradually increases. In none of the tubes is there any flocculation, the electric potential acting as a stabilizer in the weak concentration, and the solubility forces in high concentrations.

Different conditions however obtain in the red cell suspension. The

[1] This phenomenon illustrates clearly why one cannot assume that the surface of the cells is composed of albumin as well as of globulin. Since albumin would be soluble in weak concentrations of electrolyte, or even in electrolyte free sucrose, the forces of attraction between such an albumin cell surface and the water would act as a stabilizer and agglutination would be prevented. The same point is illustrated in the weaker concentrations of NaCl in experiment 7.

electric charge of its cells, as has been stated, is a transient one as contrasted to the permanent charge of the globulin or all other suspended particles. As shown in figure 7, it has fallen in all the tubes of the series to 0, and since the P.D. is the only stabilizing factor present in the weak concentration of NaCl, agglutination occurs in that region. Above M 512, however their globulin surfaces are soluble in the salt solution, and these forces of attraction between the suspending medium and the surface of the cells produce stability.

Even in this experiment, where at first glance there seems to be no similarity between the stability of the cell and globulin suspensions, on closer examination it is seen that the relations are analogous, and that the phenomena observed in the cell suspension may be explained by the hypothesis that the cell surface is composed of globulin and that suspended particles of such nature depend on either of two factors for stability, the potential difference at the interface of particle and surrounding medium or forces of attraction between the surrounding medium and the particle.

CONCLUSIONS

1. A red cell when suspended in a fluid reacts in regard to its electrokinetic properties as if it possessed a surface of globulin. A surface of either pure albumin or lipoid would not explain these reactions.

2. The electrolyte concentration of the suspending fluid determines whether the red cell suspension reacts like a suspensoid or like an emulsoid. In low concentrations (isotonic sucrose) it reacts like a suspensoid; in high concentrations (0.9 per cent NaCl) it reacts like an emulsoid.

3. These properties of the red cell suspension can be explained by the current theory of colloid stability according to which two factors operate to produce stability, the electric potential at the interface of particle and suspending medium and the forces of attraction between the suspending medium and the particle.

4. In the red cell suspension it has been demonstrated that variations in the solubility of a globulin surface in the suspending fluid account for the differences in the mechanisms which cause stability that are noted as the electrolyte concentration of the suspending fluid is changed.

BIBLIOGRAPHY

BURTON, E. F. 1921. The physical properties of colloidal solutions. 2nd ed. London and New York.

CHICK, H. 1913. Biochem. Journ., vii, 318.

KRUYT, H. H. AND H. G. DE JONG. 1922. Zeitschr. f. physik. Chem., c, 250.

LOEB, J. 1924. Proteins and the theory of colloidal behavior. 2nd ed. New York and London, 97.

MINES, G. R. 1912. Koll. Chem. Beihefte, iii, 191.

NORTHRUP, J. H. AND P. H. DE KRUIF. 1921–22. Journ. Gen. Physiol., iv, 639.
NORTHRUP, J. H. AND J. FREUND. 1923–24. Journ. Gen. Physiol., vi, 603.
OLIVER, J. AND L. BARNARD. 1924–25. Journ. Gen. Physiol., vii, 99.
ROBERTSON, T. B. 1908. Journ. Biol. Chem., iv, 1.
SHEVKY, M. C. AND D. D. STAFFORD. 1923. Arch. Int. Med., xxxii, 222.
THOMAS, A. W. 1923. Cited from WILSON, J. A. Chemistry of leather manufacture. New York, 130.

THE RATE OF ACTION OF INSULIN IN ARTIFICIALLY COOLED MAMMALS

G. J. CASSIDY, S. DWORKIN AND W. H. FINNEY

From the Laboratory of Physiology and Experimental Medicine, McGill University, Montreal

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Krogh found that under ordinary circumstances frogs develop insulin convulsions only 4 to 5 days after the injection of massive doses of insulin. This observation has been confirmed by Macleod (1924), by Huxley and Fulton (1924) and by Olmstead (1924). Huxley and Fulton (1924) further established that this period is reduced to 24 hours in frogs kept at 25°C. They concluded that "The activity of insulin itself is not essentially altered by temperature, but that its speed of action is dependent upon the metabolic rate of the animal itself." These conclusions have been corroborated by Olmstead, who reported that the onset of insulin convulsions in fishes is hastened by raising their temperature.

Olmstead (1924) also attempted to ascertain the relation between insulin convulsions and blood sugar in frogs. He found the blood sugar of normal frogs at room temperature to be very low (about 0.012 per cent), and that it rose to 0.056 per cent when the frogs had been kept at 28°C. for 2 days. At the time of convulsions, the blood sugar concentration varied between wide limits, and was often higher than it was before the administration of insulin. In view of these results, it seems impossible to correlate the blood sugar level and the occurrence of convulsions in such animals. The question whether mammals cooled to 25°C. would behave in a similar manner presented itself.

EXPERIMENTAL. For the experiments cats and dogs were used. The animals were kept on a diet of lean meat, milk and cereals for two or three weeks prior to the experiments, and were starved for the 24 hours previous to insulin injection. Amytal[1] was used as an anesthetic. This drug does not cause hyperglycemia, nor does it influence the rate of fall of blood sugar after insulin injection (Page, 1923). The animals were cooled to a temperature of 25°C. by immersing them in a cold bath. Deep rectal temperature was recorded. After removal from the bath, the animals were dried by the application of heat from electric lamps. Blood was taken

[1] Kindly supplied by the Eli Lilly Co.

from the external jugular vein in 1 cc. quantities for sugar determination by the method of Folin and Wu.

Before proceeding with the experiments on the rate of action of insulin in mammals at 25°C., it was necessary to determine the effect of lowering the body temperature upon blood sugar. Two amytalized dogs were cooled to 25°C., blood samples being taken during the process. Under these conditions there was a slight preliminary decrease in blood sugar concentration, probably due to the occurrence of vigorous shivering at this time. This was followed by a gradual rise, and, when the body temperature had reached 25°C., the blood sugar had practically returned to normal (table 1).

TABLE 1

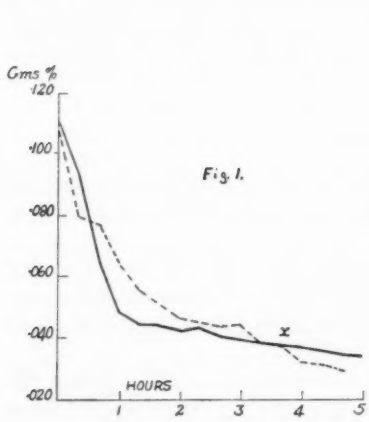
DOG 5		DOG 10	
Temperature	Blood sugar	Temperature	Blood sugar
°C.	*per cent*	°C.	*per cent*
38	0.107	38	0.111
36	0.084	32	0.094
33	0.099	30	0.106
25	0.095	24	0.113

TABLE 2

	BLOOD SUGAR BEFORE INSULIN	UNITS INSULIN PER KILOGRAM	TIME TO REACH 0.045 PER CENT	DURATION OF EXPERIMENT	BLOOD SUGAR AT END OF EXPERIMENT
	per cent				*per cent*
Dog 7	0.095	4	3 hours	5 hours 50 minutes	0.026
Dog 10	0.113	5	2 hours 18 minutes	13 hours	0.016
Dog 11	0.091	4	1 hour 30 minutes	2 hours	0.043
Dog 18	0.111	3	1 hour 20 minutes	6 hours 30 minutes	0.033
Dog 23	0.124	3	2 hours 20 minutes	3 hours 40 minutes	0.038
Cat 1		4	1 hour 35 minutes	4 hours	0.030
Cat 2		4	2 hours 20 minutes	4 hours 35 minutes	0.033

Two cats and five dogs were cooled to 25°C. under amytal anesthesia and kept at this temperature throughout the rest of the experiment by exposing them to cold air or by applying dry heat as occasion required. Immediately after removal from the bath, insulin was given subcutaneously in doses of from three to five units per kilo of body weight. Blood was withdrawn at frequent intervals. It was found that the blood sugar fell to 0.045 per cent in from 1 hour and 20 minutes to 3 hours (table 2). Since similar doses of insulin administered to normal dogs and cats after 24 hours' starvation, reduce their blood sugar to the convulsive level in from 1 to 4 hours, it would appear that the rate of fall of blood sugar is not affected by lowering of the body temperature to 25°C.

In order to obtain a more precise comparison between the rate of action of insulin at normal and at reduced body temperatures, the following experiment was performed. An amytalized dog was given 3 units of insulin per kilo subcutaneously. Blood samples were removed under aseptic conditions at 15 minute intervals for a period of 3 hours. Three grams of glucose per kilo were then injected subcutaneously to insure the animal's recovery. Three days later, this animal was cooled under amytal to 25°C., and received the same amount of insulin as before. Although the general form of the blood sugar curve on this occasion was similar to that at normal temperature, the convulsive level was reached 1 hour sooner (fig. 1).



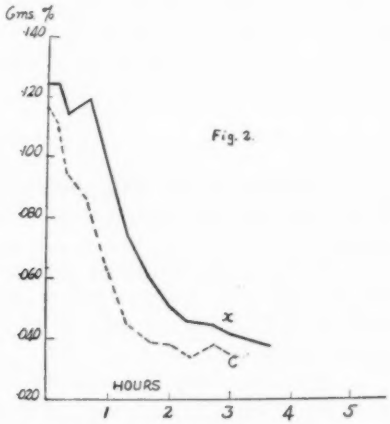


Fig. 1. Insulin curves (dog 18). Broken line—38°C. Continuous line—25°C.
Fig. 2. Insulin curves (dog 23). Broken line—38°C. Continuous line—25°C. *C*—Convulsion.

The above experiment was repeated on another dog. The curves for this animal differed from each other in two particulars: 1, the preliminary rise, which was quite marked at normal body temperature, was greatly exaggerated at 25°C.; 2, whereas the fall of blood sugar at normal temperature began about ten minutes after the insulin injection, the fall at 25°C. commenced only after 1 hour. From this point on, the curves ran almost exactly parallel.

Since the efficiency of the circulation is greatly impaired at a body temperature of 25°C., it was thought that the preliminary delay in the fall of blood sugar after subcutaneous insulin injection might have been due to slow absorption of the insulin. For this reason, a third experiment was undertaken, in which the insulin was injected intravenously. The curve for this dog at 25°C. showed a delay of 40 minutes before the fall of blood

sugar began whereas the control curve showed no delay (fig. 2). There was no preliminary rise in either case. Just as in the previous experiment, the two curves are practically parallel, once the fall commences. From this experiment the conclusion can be drawn that the preliminary delay in the fall of blood sugar at 25°C. is due to causes other than slow absorption of insulin. This delay occurred in half the animals used.

Another characteristic of the curves for animals at 25°C. is that the portion of the curves (marked *X* in figs. 1 and 2) representing the secondary fall is smoother than the corresponding part of the controls.

Seven animals remained at 25°C. for periods up to 13 hours after the injection of insulin. In no case did the blood sugar show any tendency to return to normal. On the contrary it continued to fall progressively until the end of the experiments (table 2). It has been pointed out by the authors (1925) that insulin convulsions are inhibited in dogs and cats by lowering their temperature to 25°C.

CONCLUSIONS

1. Lowering of the body temperature of a dog to 25°C. under amytal anesthesia does not appreciably change the concentration of the blood sugar.

2. In dogs and cats insulin reduces blood sugar with the same rapidity whether the body temperature be 25°C. or 38°C. In some animals, however, at 25°C. there is a delay of 40 to 60 minutes before the fall of blood sugar commences.

3. When the body temperature has been lowered to 25°C. before the injection of insulin, the form of the insulin curve for dogs does not differ appreciably from the normal.

4. When the blood sugar has been lowered by insulin in dogs and cats at a body temperature of 25°C., the sugar concentration shows no tendency to return to normal.

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BIBLIOGRAPHY

CASSIDY, DWORKIN AND FINNEY. 1925. This Journal, lxxiii, 417.
KROGH, A. Quoted by MACLEOD. 1924. Physiol. Rev., iv, 21.
HUXLEY AND FULTON. 1924. Nature, cxiii, 234.
MACLEOD, J. J. R. 1924. Physiol. Rev., iv, 21.
OLMSTEAD, J. M. D. 1924. This Journal, lxix, 137.
PAGE, I. H. 1923. Journ. Lab. Clin. Med., ix, 194.

INSULIN AND THE MECHANISM OF HIBERNATION

G. J. CASSIDY, S. DWORKIN AND W. H. FINNEY

From the Laboratory of Physiology and Experimental Medicine, McGill University, Montreal

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The hibernating mammal presents several unique features as regards metabolism. The change from the homoiothermic to the poikilothermic condition and vice versa is of considerable interest. Numerous theories have been advanced to explain these transitions, and frequent attempts have been made to induce artificial hibernation, but, so far as we know, the essential problem is still unsolved. A good account of previous work on this subject is contained in an article by Rasmussen (1916). Lowering of external temperature, depression of respiration by cold, deficient food supply, cerebral anemia, auto-narcosis, acapnia, and altered function of the endocrine system have all been cited as primary causes, but experiment has amply shown that not one of these is in itself sufficient to produce hibernation. Thus, animals that normally hibernate, have been known to become torpid even in mid-summer and in the presence of a rich food supply; and cerebral congestion has been reported in animals during hibernation.

Mere lowering of body temperature was regarded as sufficient to induce artificial hibernation by Simpson (1902), who used monkeys, and by Simpson and Herring (1905), who used cats. The last named workers claimed that when the rectal temperature falls to 24°C. the heat-regulating mechanism does not assert itself and the temperature of the animal tends to become the same as that of its suroundings. This conception has been disproved by Britton (1922) who showed that cats reduced to a deep rectal temperature of between 24° and 22°C. effect spontaneous recovery in six to seven hours, and that these animals can recover from a temperature of between 21° and 19°C. in ten to twelve hours. Tait and Britton (1923) using the woodchuck (*Arctomys monax*) have further established the fact that an animal which naturally hibernates recovers spontaneously and promptly after artificial cooling to temperatures varying between 12° and 3°C.

Dubois (1909), (1901) produced a condition of inactivity by causing his animals (marmots and dogs) to breathe air containing a large proportion of carbon dioxide. The animals, however, did not assume the characteristic poikilothermic state.

Finally, one might quote several observations which would indictate the existence of a relationship between some of the ductless glands and hibernation. Cushing and Goetsch (1913) noted that excision of the pituitary in man and also in animals produces symptoms comparable to hibernation. These authors corroborated the findings of Gemelli (1906), who observed marked changes in the chromophile cells of the anterior lobe of pituitary glands removed from marmots in the hibernating state. Mann (1916) also reported such changes in the gopher, but they were not constant. He found gross as well as microscopical seasonal changes in the adrenals, and in the glands of actually hibernating animals noted a general shrinkage and vacuolization of the cells, especially in the zona granulosa and upper part of the zona fasciculata. The only other changes of interest were in the islets of Langerhans in the pancreas, which resembled those of a resting gland.

The characteristics of the true hibernating state are: unconsciousness, inactivity, and a fall in temperature to within a degree or two above that of the environment; the respiratory rate is slowed to about one per minute or even less; the heart rate is likewise decreased. Further, it has been shown by Dubois (1894) and by Rasmussen (1915) that the carbon dioxide content of the blood in the dormant animal is abnormally high. While the metabolic rate is greatly reduced, there is evidence of a remarkable change in the character of the nutrition. During hibernation there is a gain in weight, Pembrey (1901) reporting that three marmots gained during one hour 11, 8 and 17 centigrams respectively. Reiset and Regnault (1849) recorded a gain of 5.9 grams in five days in the same animal. This is accompanied by a decreased ratio of carbon dioxide output to oxygen intake, both of which are greatly decreased, and by diminished excretion of water as compared with that of the active animal. On the transition to the active state there is marked shivering and a sudden rise of the respiratory quotient; the animal's temperature rapidly rises, that of the dormouse mounting from 13.5° to 35°C. in one hour, and within a very short time the usual mammalian temperature level is reached. It was found by Claude Bernard, by Voit (1878) and by Dubois (1899) that the liver of the hibernating animal contains much glycogen, which disappears on awakening. Pembrey (1901) correlating this observaton with the fact that the respiratory quotient of the hibernating animal is very low (0.53), concluded that during torpidity fat is being continuously converted to glucose, which is stored as glycogen, and reconverted to glucose for utilization during awakening, when the respiratory quotient rises (0.71 to 0.89).

The only available record of the blood sugar in the hibernating mammal is that of Dubois, who found it to be 0.009 per cent in the European marmot; in the active animal it was 0.117 per cent. In the light of Pembrey's conclusions this finding naturally suggests some correlation between

hibernation and the concentration of the blood sugar. One might further quote Dubois (1894) to the effect that extirpation of the ganglia of the solar plexus or ligature of the portal vein and of the inferior vena cava just above the liver prevents the remarkable rise in temperature seen in the awakening marmot. Additional evidence for some such relationship is found in the fact that Mills (1892) and Simpson (1912) could prevent woodchucks from hibernating by providing them with an adequate supply of food, a fact also observed in this laboratory during the last two winters. The blood sugar of one of these animals was determined on two occasions and was found to be 0.361 per cent and 0.235 per cent (no anesthetic was used in removing the blood). This woodchuck was given insulin and developed typical convulsions in two hours. Blood taken immediately after a convulsion contained 0.057 per cent glucose. We concluded from this experiment that, if the figure of 0.009 per cent for the dormant animal is characteristic, some mechanism must come into play during hibernation to prevent the occurrence of hypoglycemic convulsions. Accordingly it was decided to try the combined effect of lowered body temperature and of insulin hypoglycemia in order to see whether we could obtain some further data regarding the mechanism of hibernation.

METHOD. Cats and dogs were used for the experiments, a sufficient number of woodchucks not being available. The animals were kept on a diet of lean meat, milk and cereals for a week or two prior to the experiments. The temperature of the animals was lowered by immersing them in a cold bath. An anesthetic was necessary to prevent struggling during cooling. Amytal was used, since it neither causes hyperglycemia nor interferes with the fall of blood sugar following insulin administration. Preliminary control experiments clearly demonstrated that, in doses used by us, amytal does not prevent insulin convulsions in normal animals. Some of the experiments were carried out without anesthesia; these will be referred to later. Deep rectal temperature was recorded, the bulb of the thermometer being inserted to a depth of about 8 cm. Blood was removed from the external jugular vein in 1 cc. quantities for sugar determination by the method of Folin and Wu. After removal from the bath the animals were completely dried by the application of dry heat, in order to prevent loss of heat by evaporation. The average weight of the cats was 3 kilos, that of the dogs 8 kilos. The insulin was given subcutaneously in doses of from 3 to 5 units per kilo of body weight.

In another communication (1925) we have pointed out that lowering of the body temperature of a dog or cat to 25°C. does not appreciably affect the rate at which insulin reduces blood sugar.

INHIBITION OF INSULIN CONVULSIONS BY LOWERED BODY TEMPERATURE. After having been cooled to a temperature of 25°C. under amytal anesthesia, each animal was removed from the bath. Insulin was then injected

and blood taken at frequent intervals in order to determine the moment at which the blood sugar reached the level of 0.045 per cent. In the meantime artificial measures were taken to keep the body temperature between 24° and 26°C.

TABLE 1

	BLOOD SUGAR BELOW 0.045 PER CENT		REMARKS
	Time kept at 25°C.	Time to raise temperature	
Cat 1	2 hours 25 minutes	2 hours 15 minutes	Animal warmed. Premonitory signs at 35.0°C. followed by tonic convulsions 60 minutes later. Blood sugar after convulsions—0.020 per cent
Cat 2	2 hours 15 minutes	3 hours 25 minutes	Animal warmed. Premonitory signs at 28.5°C. Tonic convulsions at 37.3°C. Blood sugar after convulsion, 0.014 per cent
Cat 5	2 hours 25 minutes	2 hours 50 minutes	Animal warmed. Premonitory signs at 32°C. Weak convulsion at 37°C. followed by several tonic convulsions at 38.5°C. Blood sugar after convulsions 0.023 per cent
Dog 7	2 hours 50 minutes	3 hours 5 minutes	Animal warmed. Premonitory signs at 38°C. followed by tonic and clonic convulsions 10 minutes later. Blood sugar after convulsions 0.026 per cent
Dog 10	10 hours 30 minutes	Not warmed	No convulsions. Blood sugar at death 0.016 per cent
Dog 17	8 hours	Not warmed	No convulsions. Blood sugar at death 0.036 per cent
Dog 18	5 hours 19 minutes	Not warmed	No convulsions
Cat 11	2 hours 46 minutes	Not warmed	No convulsions

As shown in table 1, these animals remained at a temperature of about 25°C. and at a blood sugar concentration considerably below 0.045 per cent for periods ranging from 1 hour and 45 minutes to 10 hours and 30 minutes. No convulsions were observed in any instance under these conditions. The temperature of the first four animals was then raised by the application of dry heat. At or about body temperature all four showed convulsions. These were in every case preceded by premonitory signs (jerky inspira-

tions, tremors and muscular twitchings), in one case at a temperature as low as 28°C. (cat 2). The convulsions were sometimes tonic; sometimes tonic followed by clonic. The blood sugar fell progressively until the end of the experiments, reaching 0.014 per cent in one case (cat 2). The occurrence of premonitory signs at 28°C. suggests the possibility that convulsions might supervene if an animal were left for a sufficient length of time at this temperature. Two insulinized dogs were kept at 25°C. for 8 hours and for 10½ hours respectively, without showing any convulsive symptoms, although the blood sugar was below the convulsive level during the entire time (dogs 17 and 10).

Before concluding that insulin convulsions are inhibited by mere fall of body temperature, it was necessary to show that amytal anesthesia is not the determining factor. Experiments on non-anesthetized animals were accordingly attempted. A cat (no. 5) was cooled without anesthesia to 25°C., and insulin was injected. Although the blood sugar concentration remained below 0.045 per cent for 2 hours and 25 minutes at this temperature, no convulsions occurred. Warming was then commenced, and 2 hours and 50 minutes later, when the body temperature had reached 38.5°., strong tonic convulsions were exhibited. These were preceded by premonitory signs at 32°C. and by weak convulsions at 37°C. (cat 5). Since the behavior of this cat was the same as that of the amytalized animals, the conclusion can be drawn that insulin convulsions were prevented in these animals by mere lowering of body temperature.

Cats were used because it was impractical to cool dogs to 25°C. without anesthesia. Even then, considerable difficulty was experienced. Three animals died in convulsions, either during immersion in the cold bath, or soon after removal, without having received insulin. They all showed a hyperglycemia, varying from 0.224 per cent to 0.440 per cent, and a decreased carbon dioxide combining power of 38.7 to 19.7 volumes per cent. This reaction on the part of cats is brought forward as an explanation of one of our experimental results—the only case in which convulsions were observed following insulin administration at low body temperature. This particular non-anesthetized cat remained in a hypoglycemic condition for one hour, when it suddenly exhibited a weak, though definite, tonic convulsion followed by several strong clonic convulsions. A Van Slyke determination showed a decreased alkali reserve of 27.1 volumes per cent. The animal was given glucose intravenously, and five minutes later, when the blood sugar was 0.500 per cent, again passed into convulsions. Hence, these convulsions can be ascribed to causes other than insulin hypoglycemia.

Our results in connection with inhibition of convulsions at lowered body temperature are in line with those of Krogh. This observer found that administration of large doses of insulin to mice rarely causes convulsive symptoms, but that the animals show great muscular weakness and pass into a comatose condition with a fall of body temperature to a very low level. He also noted that placing the animals in an incubator at 28°C.

causes convulsions of the ordinary type to supervene. We are as yet unable to state whether convulsions in our animals were prevented or only greatly delayed by the reduced body temperature. Krogh has reported that under ordinary circumstances frogs develop convulsions only four to five days after massive doses of insulin, a fact confirmed by Huxley and Fulton (1924) and by Olmstead (1924). Further, Huxley and Fulton have demonstrated that frogs pass into convulsions in twenty-four hours when their temperature is raised to 25°C. Their findings in this regard have been corroborated by Olmstead, who also showed that fishes behave in a way similar to frogs with respect to the relation of body temperature and time of onset of insulin convulsions. Whether mammals cooled to 25°C. would show convulsions if left for a sufficient length of time at the convulsive level, is still an unsettled question.

HYPOGLYCEMIA ABOLISHES SHIVERING. In the course of the above experiments it was noted that, when the blood sugar fell to 0.045 per cent or slightly above this level, the animals ceased to shiver. During the whole of the cooling process they shivered strongly, even when their temperature was 25°C. The shivering was continuous at first, but as the temperature fell it occurred only with inspiration. One and a half to three hours after the injection of insulin, when the blood sugar had reached a level of 0.063 per cent to 0.053 per cent, shivering was markedly diminished. Soon it ceased altogether; at this point the blood sugar varied from 0.052 per cent to 0.034 per cent, the most common figure being 0.045 per cent (table 2). Once abolished, the shivering reflex never returns spontaneously. In the animals that were subsequently warmed, no shivering whatsoever was observed, even as the normal temperature level was approached. The first signs of movement were muscular tremors, not related to any particular phase of the respiratory cycle, and these were soon followed by convulsions. No shivering was observed during a period of 10 hours and 45 minutes in an animal left at 25°C. with a low blood sugar (dog 10).

This behavior is in marked contrast to the reaction shown by the non-insulinized animal. A dog or cat with a normal blood sugar shivers as soon as it is exposed to cold, and continues to shiver during the entire time that its temperature is below the normal level. Thus, one cat (no. 3) used as a control was anesthetized like the rest, and cooled to 25°C., but was not given insulin. It continued to shiver vigorously after removal from the bath, and in a short time had raised its own temperature to 30°C. and was able to move about freely.

Whether hypoglycemia is the essential factor in the abolition of the shivering reflex could be determined only by the injection of a glucose solution. Accordingly six animals were given glucose after this reflex had disappeared. In two of these, intravenous injections induced shivering imme-

diately—indeed before the injection was completed. In other cases, where subcutaneous injections were given, shivering came on gradually. It was observed first in the flank, then in the gluteal region and shoulder muscles. The lower and upper limbs were next involved. Soon the shivering became general and increasingly vigorous as the temperature rose. One animal, having recovered its ability to shiver, was again given insulin (10 units).

TABLE 2

	TEMPERATURE WHEN SHIVERING CEASED	BLOOD SUGAR WHEN SHIVERING CEASED	GLUCOSE INJECTION	REMARKS
	°C.	*per cent*		
Cat 1	25.0	0.040	No glucose	Warmed to 38.6°C. No shivering during warming
Cat 2	24.5	0.040	No glucose	Warmed to 38.5°C. No shivering during warming
Cat 3	Control			Cooled to 25°C. then warmed to 39°C. shivering throughout
Cat 5	26.0	0.034	No glucose	Warmed to 38°C. No shivering during warming
Dog 12	35.0	0.044	No glucose	Warmed to 38°C. No shivering during warming
Dog 10	26.2	0.053	No glucose	No shivering during 10 hours 45 minutes following cessation
Cat 9	25.0	0.036	Glucose intravenously	Shivering began immediately
Cat 11	26.3	0.043	Glucose subcutaneously	Shivering began 5 minutes later
Dog 18	38.0	0.045	Glucose intravenously	Shivering began immediately
Dog 18	25.2	0.045	Glucose subcutaneously	Shivering began 9 minutes 5 seconds later
Dog 19	35.4	0.052	Glucose intravenously	Shivering began 2 minutes 15 seconds later
Dog 20	25.5	0.050	Glucose subcutaneously	Shivering began 6 minutes later
Dog 21	37.5	0.043	Glucose subcutaneously	Shivering began 1 minute later. No amytal

The shivering soon became less violent, and 1 hour and 45 minutes later had once more disappeared. The possibility that the restoration of shivering was due to a difference in temperature between the injected solution and the animal's body, was disposed of by previously injecting Ringer's solution intravenously at a temperature of 11°C. This had no effect; whereas glucose given 8 minutes later under similar conditions caused the animal to shiver at once.

Later we were able to demonstrate that hypoglycemia prevents shivering in non-anesthetized dogs at normal temperature. A wet dog placed out-of-doors for a few minutes (external temperature 7°C.) began to shiver immediately. The animal was returned to the room and given insulin. Two hours and fifteen minutes later there was no shivering on exposure to cold, even after the body temperature had fallen 2°C. The blood sugar at this time was 0.043 per cent. Glucose was then injected subcutaneously and shivering began about one minute later (dog 21). The amytalized animal at normal temperature behaves similarly.

A STATE SIMULATING HIBERNATION. Cats and dogs at a body temperature of 25°C. spontaneously regain their normal temperature unless actively cooled. At or about the time shivering ceases in the insulinized animal, this power is lost. Until the blood sugar reaches a level of about 0.063 per cent, shivering is still active, and the animal's temperature rises slowly. With the diminution of shivering (blood sugar 0.063 per cent to 0.053 per cent) the rise is checked, and the temperature remains constant for a short time. This is succeeded by a gradual fall to a level within a degree or two above the temperature of the room, at which point it remains constant for a number of hours. Eventually the animal's temperature again declines and, before death occurs, may fall to that of the surroundings (fig. 1).

The tendency of the temperature to remain at a low level was demonstrated in seven experiments. In two cases the condition was terminated by artificial warming. In three cases glucose was injected, with a consequent rise in temperature due to shivering. Two animals (dogs 17 and 10) were allowed to remain without interference in this state for 13 hours and for 10 hours and 45 minutes respectively, at the expiration of which time death ensued. These last showed no tendency to raise their temperature.

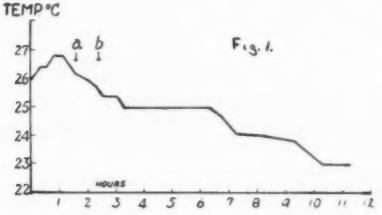
While the onset of the condition described above may occur at somewhat variable blood sugar levels, it is intimately connected with the disappearance of shivering. From this time on the animal presents a marked contrast to its previous condition. A cat cooled to 25°C., even under light amytal anesthesia, is conscious and carries out active purposeful movements. When the blood sugar concentration has fallen to 0.045 per cent following insulin administration the animal while retaining the same temperature, is no longer conscious or active, and is insensible to painful stimulation. The corneal reflex is usually absent and the pupils are dilated. The knee-jerk is still active. With the exception of those of respiration, no body movements are perceptible. The respiratory rate varies from five to eighteen per minute. No cyanosis is evident, for with the slowing in respiratory rate there is a compensatory increase in depth.

In one instance Biot breathing was observed (dog 17); periods of respiration lasting 20 seconds alternated with periods of apnea lasting 65 seconds. Each group consisted of from three to five respirations of about equal depth, the onset and cessation

being abrupt. These time relations remained constant for 45 minutes, at the end of which time the animal died. Insertion of the thermometer into the rectum caused an increase in both rate and depth of respiration. This reflex was last elicited twenty minutes before death.

With the cessation of shivering the heart rate also decreases. In one animal it diminished from 73 to 56 per minute, in another from 45 to 36. A continuous slowing in rate is observed until death (table 3). This finding presents a contrast with the insulinized animal under amytal at normal body temperature, in which Edwards and Page (1924) found the heart rate to be accelerated.

When glucose is administered a change is observed almost at once. With the onset of shivering the animal's temperature begins to rise; there are signs of returning consciousness and spontaneous body movements occur. In a non-anesthetized cat (no. 9), whose temperature had pre-



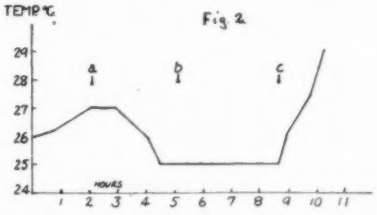


Fig. 1. Temperature curve (dog 10) after insulin; no subsequent injection of glucose. Cf. table 3. *a*, Shivering diminished; *b*, shivering stopped.

Fig. 2. Temperature curve (cat 9) after insulin, showing effect of subsequent glucose injection. *a*, shivering diminished, blood sugar 0.051 per cent; *b*, shivering stopped (0.038); *c*, glucose injection.

viously remained at 25°C. for 4 hours and 8 minutes, there was a rise of 4° in 1 hour and 25 minutes (fig. 2). Fifteen minutes after the injection the animal was fully conscious and active.

Up to the present the greatest duration of life observed by us in cooled insulinized animals after the disappearance of shivering has been 13 hours.

Discussion. While it would be premature to conclude that the state induced in these animals is identical with true hibernation, nevertheless several features are common to the two conditions: 1, the tendency of the animal to assume the temperature of its surrounding; 2, the absence of shivering and of spontaneous movements; 3, loss of consciousness; 4, profound reduction in the rate of metabolism; 5, a prolonged state of hypoglycemia without convulsions.

The changes brought about by injection of glucose into the blood stream resemble those observed in the awakening marmot. Both respiration and heart rate are quickened; there is violent shivering, and just as Horvath

(1878) has remarked of the marmot, when once the shivering movements have commenced, nothing can prevent the animal from awakening and its temperature from rising. For example, one non-anesthetized cat (no. 9) from a state of dormancy recovered full consciousness in a short time, and would have regained its normal temperature if allowed to do so.

It is plain that in this transition glucose plays the important part. Here we may again adduce the conclusions of Pembrey that during the awakening of the marmot the metabolism is that of carbohydrate combustion.

When the temperature of an animal has fallen, recovery of normal temperature depends in great part upon the capacity for shivering. This in turn, is determined by the concentration of the blood sugar. When the

TABLE 3

Dog 10, weight 5 kgm., amytal anesthesia

TIME	TEMPERATURE OF DOG	TEMPERATURE OF ROOM	HEART RATE	RESPIRATORY RATE	BLOOD SUGAR	REMARKS
	°C.	°C.			per cent	
7.08 p.m.	26.0	23.0	79	8	0.113	45 units insulin injected
8.30	26.8	20.0			0.081	Strong shivering
9.00	26.5				0.064	Shivering was diminished
9.15	26.2		73	7	0.053	Shivering stopped
9.30	26.0				0.043	
10.15	25.4	23.5	56	6	0.037	
11.00	25.0	22.0	54	18	lost	
12.30 a.m.	25.0	24.0	54	18	0.028	
2.00	25.0	23.5	48	16	0.023	
2.26	24.7	23.5	46	18		
3.00	24.1	24.0	48	17	0.021	
4.00	24.0	23.5	39	10	0.018	
5.00	23.8	22.5	40	8	0.018	
7.00	23.0	23.0	38	5	0.016	
8.00	23.0	23.0	38	5		
8.00–9.00						Animal died

blood sugar level is below 0.045 per cent, the function of the heat-regulating mechanism is markedly impaired if not completely inhibited.

Whether liver metabolism or muscular activity is the chief source of increased heat production in the awakening animal has been a matter of debate. The view of Dubois already quoted is that the liver plays the important part, but the observations of Pembrey and White (1896) and of Pembrey (1901) indicate that the chief cause of the rise in temperature is the increased metabolism of the muscles. In our opinion the latter view is correct. The findings of Dubois may have been due to the fact that he interfered with the glucose output of the liver. The observations of Claude Bernard, of Voit and of Dubois, may once more be cited, to the

effect that during hibernation the liver contains much glycogen, which it loses as the temperature rises upon awakening.

While we cannot claim to have produced actual hibernation, inasmuch as our experiments have been carried out on dogs and cats, it seems reasonable to suppose that if similar experiments were carried out on animals which normally hibernate, they would result in the production of artificial hibernation and of awakening. Such experiments are now in progress in this laboratory.

CONCLUSIONS

1. Lowering of the body temperature of cats and dogs to 25°C. inhibits insulin convulsions.

2. Lowering of the blood sugar by means of insulin abolishes the shivering reflex. Administration of glucose causes its reappearance.

3. The combined action of cold and insulin in cats and dogs produces a state simulating hibernation. Subsequent injection of glucose restores the animal to normal.

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BIBLIOGRAPHY

BERNARD, C. Quoted by PEMBREY (1901).
BRITTON, S. W. 1922. Quart. Journ. Exper. Physiol., xiii, 1, 55.
CASSIDY, DWORKIN AND FINNEY. 1925. This Journal, lxxiii, 413.
CUSHING AND GOETSCH. 1915 Journ. Exper. Med. xxii, 25.
DUBOIS, R. 1894a. Compt. rend. soc. biol., ser. x, i, 821.
1894b. Compt. rend. soc. biol., 36, 115.
1899. Journ. de Physiol. et de Path. gen., ii, 1020.
1901. Compt. rend. soc. biol., liii, 231.
1909. Compt. rend. Acad. Sci., cxlviii, 1787.
Quoted by BIERRY AND FANDARD. 1912. Compt. rend. Acad. Sci., cliv, 1717.
EDWARDS AND PAGE. 1924. This Journal, lxix, 177.
GEMELLI, A. 1906. Arch. per le scienze mediche, xc, 941.
HORVATH, A. 1878. Verhandl. d. phys. med. Gesellsch., Würzburg, xii, 139.
HUXLEY AND FULTON. 1924. Nature, cxiii, 234.
KROGH, A. Quoted by MACLEOD. 1924. Physiol. Rev., iv, 21
MANN, F. C. 1916. This Journal, xli, 173.
MILLS, W. 1892. Trans. Royal Soc. Canada, 49.
OLMSTEAD, J. M. D. 1924. This Journal, lxix, 137.
PEMBREY, M. S. 1901. Journ. Physiol., xxvii, 66.
PEMBREY AND WHITE 1896. Journ. Physiol., xix, 477.
RASMUSSEN, A. T. 1915. This Journal, xxxix, 20.

RASMUSSEN, A. T. 1916. Amer. Naturalist, l, 609.
REGNAULT AND REISET. 1849. Ann. de chim. et phys., Paris.
SIMPSON, S. 1902. Journ. Physiol., xxviii, Proc. Physiol. Soc., xxxvii.
SIMPSON, S. 1912. Proc. Soc. Exper. Biol. and Med., ix, 92.
SIMPSON AND HERRING. 1905. Journ. Physiol., xxxii, 305.
TAIT AND BRITTON. 1923. Quart. Journ. Exper. Physiol., supplementary vol., 226.
VOIT. 1878. Zeitschr. f. Biol., xiv, 57.

SOME PHYSIOLOGICAL EFFECTS OF HOT-AIR BATHS

CORNELIA GOLAY BENEDICT, FRANCIS G. BENEDICT
AND EUGENE F. DU BOIS

From the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts, and the Russell Sage Institute of Pathology in affiliation with the Second Medical Division of Bellevue Hospital, New York City

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As a natural result of studies at the Nutrition Laboratory on the metabolism of individuals immersed in neutral water baths (Benedict and Benedict, 1924) the possibility of studying the metabolism of individuals in a current of hot, desiccated air, such as presented itself in certain experiments being made in the Second Medical Division of Bellevue Hospital in the spring of 1924, immediately challenged our attention. Mr. O. Newhouse had devised and installed in Bellevue Hospital a machine capable of delivering into a bag or receptacle large volumes of rather highly desiccated air at varying temperatures. Into this receptacle the nude human body could be placed (the head being exposed) and bathed in a rapidly moving current of heated air. Verbal reports with regard to certain tests of the apparatus indicated that extraordinarily high temperatures of the air prevailed without discomfort to the subject.

Owing to the difference in the specific heat of air and water, the effect of hot air on the loss of heat or, indeed, the metabolism of human subjects, even if nude, could not be predicted from experiments on subjects immersed in water at various temperatures. In the literature a number of observations on the physiological effects of extremely hot air have been recorded,[1] but modern technique has made it possible to determine more definitely the influence of hot-air baths upon body temperatures (mouth, rectal, vaginal and surface temperatures) and the gaseous metabolism. In planning these hot-air experiments, therefore, it was our purpose to include as many physiological measurements as possible under the rather restricted conditions of having the body of the subject tightly enclosed in a large oilskin bag.

APPARATUS AND TECHNIQUE. *The hot-air machine: Apparatus for control of the air.* A rotary, positive-pressure, air blower, directly connected with an electric motor and regulating rheostat, conducted air

[1] Pembrey (1898) has given an excellent résumé of the early work on the effects of hot air in his article on "Animal heat."

first through about 9 kgm. of strong sulphuric acid, and then through a tube containing an electric heating unit. The heated air leaving the machine was conducted through a 3-meter length of flexible metal tubing, finally issuing in a nozzle 5 cm. in diameter, which could be connected with the bag in which the subject was placed. Anemometer tests showed a constant air discharge into the bag of 500 liters of air per minute.

Temperature of the air. The true significance of the amount of water vapor in the air cannot be considered independent of the temperature. Actual tests of the temperature of the air *entering the bag* showed that it was usually about 80° to 85°C. On the evening of March 18 new heating units were placed in the apparatus, so that the next morning the temperature of the air was somewhat higher. On March 20, due to a defective connection, a distinctly lower heating effect was noted by the subject, and the temperature of the air was found to be but 75°C. instead of the usual 85°C. On April 10, after repairing the circuit, a further test was made in which it was seen that the temperature of the air entering the bag ranged about 90°C. Physiologically, we are interested solely in the actual temperature of the air entering the bag, and this, as stated above, was frequently observed in our experiments and was usually not far from 80° to 85°C.

The enclosing bag. The bag itself, in which the nude subject was placed, was made of finely-woven cotton cloth, impregnated with a filler somewhat like that used for waterproof coats commonly called "oilskins." This bag, when laid flat, was 88 cm. wide and 225 cm. long. At the top there was a 34-cm. slit with a collar attached to it, with a draw-string to be used for closure round the subject's neck. There were likewise two slits or openings, both provided with rather long collars, to which the air pipes could be connected, each slit being 8 cm. wide. The air entered the bag through one opening near the right foot and left the bag through the other opening near the left shoulder of the subject. The entire bottom of the bag was left open, but by folding the bottom part on itself in two or three folds and clamping the folds between wooden clamps, a tight closure could be obtained. When the apparatus was in use the bag became greatly distended, thus surrounding the subject with an atmosphere of hot air. While the bulk of the air was supposed to leave through the opening over the left shoulder, undoubtedly there were considerable losses by leakage around the neck, as it was impossible to draw the string tightly enough around the collar without much discomfort to the subject.

Special physiological methods. A standard Benedict-Collins (1920) portable respiration apparatus was used with two of the three subjects whose basal metabolism was measured. The experiments with the third subject, Dr. D. B., were made with the apparatus as modified by Roth (1922). Wilson soda-lime was used, and the measurement of the oxygen

consumption was based, as usual, upon the contraction of the spirometer bell, corrected for change in temperature of the bell and reduced to 0°C. and 760 mm. pressure. All records were made upon a kymograph, including a base line and a time line. The kymograph tracings furthermore gave the respiration rate, respiratory volume, and the apparent volume of oxygen absorbed. The pulse was usually taken at the wrist. The skin temperature and the rectal and vaginal temperatures were taken by thermo-junctions, commonly used in this Laboratory (Benedict, Miles and Johnson, 1919). The galvanometer readings were made on a small Leeds and Northrup portable galvanometer, and each millimeter deflection corresponded to about 0.06° to 0.08°C. Mouth temperatures were taken by a standard mercury thermometer. The body-weights were determined upon a platform scale reading to about 10 grams.

Subjects and experimental routine. The physical characteristics of the 5 subjects used are given in table 1. The subject entered the laboratory

TABLE 1

Height, weight and age of subjects

SUBJECT	OCCUPATION	HEIGHT	NUDE WEIGHT	AGE	
		cm.	*kgm.*	*years*	*months*
Mr. D.	Medical student	181	83.4	27	9
Mr. E.	Medical student	170	70.0	29	5
Dr. D. B.	Physician	180	75.0	41	9
Mrs. F.	Patient	161	105.4	30	
Miss G.	Masseuse	171	90.8	33	6

in a post-absorptive condition and, after disrobing and having the nude weight recorded, immediately went to bed, well covered with bedding. After a half-hour period of repose, several basal metabolism measurements were made. The subject then entered the bag and after hot air had been discharged for some minutes over the body, numerous respiration experiments were made. Studies of the skin temperature were frequently made before and after the subject entered the bag. At the conclusion of the experiment the loss of weight was recorded.

Metabolism experiments were made with Mr. E., Mr. D. and Dr. D. B. Surface-temperature measurements were taken on Mr. E., Mr. D., Mrs. F. and Miss G. Rectal temperatures were taken with all the male subjects. With the first two subjects these were made continuously, with the last, Dr. D. B., intermittently. Vaginal temperatures were taken in the case of Miss G.

We wish herewith to express our appreciation of the skill of Doctors Holt and Sutliff, who conducted the observations on Dr. D. B.

STATISTICS OF EXPERIMENTS. *Experiment I* (*March 16, 1924*). Mr. E. arrived without breakfast and lay down on the bed, well covered with blankets, at 9:10 a.m. His nude weight was 69.63 kgm., and the room temperature *circa* 17°C. The pulse rate at 9:21 a.m. and 9:42 a.m. was 51 beats per minute in each case. Respiration experiments were made in three periods at 10:08 a.m., 10:42 a.m. and 11:00 a.m. Skin-temperature measurements were made at 11:12 a.m. The pulse was recorded between periods and varied only from 48 to 51 beats per minute. The measurements of oxygen consumption and other physiological data are given in the general tables under subject E. At 11:25 a.m., after voiding, his nude weight was 69.3 kgm. He entered the bag at 11:34 a.m., and the hot air was turned on. Four respiration experiments, each of about 10 to 12 minutes, were made while the subject was inside the bag. During the last six minutes of the last period the heating resistances were cut off and the air passing over the body was much nearer room temperature. After leaving the bag at 1:20 p.m., the body-weight was 68.90 kgm.

Experiment II (*March 17, 1924*). Mr. E. came to the hospital without breakfast and his nude weight was 70.0 kgm.; room temperature *circa* 16°C. He lay down at 9:09 a.m., well covered with blankets. The pulse was 54 at 9:25 a.m., 51 at 9:36 a.m., and 55 at 10 a.m. Basal metabolism measurements were made at 10:08 a.m. and 10:37 a.m. Skin-temperature measurements were taken at 10:58 a.m. After voiding urine at 11:06 a.m., when the weight was 69.75 kgm., he entered the bag, which was covered with a blanket. The air was on at 11:14 a.m. Respiration experiments were made at 11:35 a.m., 12 m., and 12:28 p.m. The results for the respiration experiments are given in the general tables. After the subject left the bag, the body-weight was 69.25 kgm.

Experiment III (*March 17, 1924*). To secure further information with regard to the influence of a hot-air bath upon basal metabolism, a case of obesity was studied, to see what rôle the protective layer of fat would play. The subject was a very fat woman, Mrs. F., a patient in the hospital, afflicted with arthritis with a special localization in the right ankle. The ward record showed that she had a rectal temperature of 37.4°C., and a pulse rate of 92 beats per minute. Metabolism measurements could not be made, as she was untrained to the apparatus. Mrs. F. rested for nearly an hour in the laboratory bed, covered with a bathrobe and a blanket, after which a survey of the skin temperature was taken under the clothing. She then entered the bag, nude, at about 3:25 p.m. The hot air was turned on at 3:30 p.m. and a series of skin-temperature measurements were made at 3:45 p.m., at 4:01 p.m. and at 4:15 p.m. The temperature in the mouth at 3:40 p.m., about 15 minutes after entering the bag, was 36.9°C. and the subject was freely perspiring under the arms. The pulse at 3:52 p.m. was 116, at 4:07 p.m. it was 112 and at 4:20 p.m. again 116. The heat was turned off at 4:15 p.m. and the subject left the bag at 4:30 p.m. The final weight was 104.7 kgm.

Experiment IV (*March 18, 1924*). Mr. D., with a nude weight of 83.35 kgm., came to the laboratory as usual without breakfast and lay down on the bed at 9:12 a.m., nude, under bedding, with a hot-water bottle at his feet. The temperature of the room was very low, i.e., 12°C. Basal metabolism measurements were made at 10:05 a.m., 10:28 a.m. and 10:43 a.m. At 10:24 a.m., when the pulse rate was 81 and the room temperature about 16°C., the hot-water bottle was removed. At the end of the third period a series of skin temperatures was taken. At 11:10 a.m., the weight after voiding was 83.02 kgm. He entered the bag, which was covered with a blanket, at 11:14 a.m., and hot air was turned on at 11:21 a.m. Respiration experiments were made at 11:40 a.m., 12:04 p.m. and 12:35 p.m. At 12:21 p.m. a series of skin temperatures was taken. The hot air was turned off at 12:50 p.m. The subject left the bag at 1:09 p.m., when he weighed 82.41 kgm.

Experiment V (March 18, 1924). Miss G., a nurse (nude weight 90.8 kgm.), went to bed at 1:25 p.m., in bathrobe and well-covered with blankets. Mouth temperature was 36.8°C. With this subject we were able not only to compare the skin temperatures but to make vaginal-temperature records with special reference to the temperature gradient between the interior and the exterior of the body. A series of skin-temperature measurements was made while the subject was in bed and under the clothing at 1:58 p.m. Her pulse at this time was 78. She entered the bag at 2:30 p.m.; hot air turned on at 2:39 p.m. At 3:52 p.m. the pulse rate was 112. At 2:56 p.m. the subject was moist all over the surface of the body, and at 3:20 p.m. she was beginning to perspire on the face. Vaginal temperatures were recorded during the entire sojourn in the bag. Skin temperatures were recorded at 1:58 p.m., at 3:13 p.m. and again at 3:42 p.m. At 3:56 p.m. the hot air was cut off and at 4:10 p.m. hot air was put on again, but no further observations were made on the subject, and she left the bag at 5 p.m., weighing 89.93 kgm.

Experiment VI (March 19, 1924). At 8 a.m. Mr. E. had a cup of caffeine-free coffee with saccharin and about 30 cc. of cream. His nude weight was 69.5 kgm. Mouth temperature 36.1°C. Room temperature at 10 a.m., 17.1°C. He lay down at 9 a.m. and at 9:45 a.m. the pulse was 55. A change had been made in the heating units used in the hot-air apparatus, and thus the air probably entered the bag at a temperature of about 95°C. Respiration experiments were made at 10:02 a.m. and at 10:25 a.m. At 11 a.m. his weight after voiding urine was 69.20 kgm. He entered the bag and hot air was turned on at 11:04 a.m. The temperature between the bag and a blanket at the air-intake point was 68°C., and between the bag and the blanket at exit was 37.4°C. at 11:35 a.m. At 11:41 a.m. and 12 m. respiration experiments were made. The bottom of the bag was too hot for the operator to touch by hand, and the subject was disposed to keep his feet as far away from the intake as possible. At 12:25 p.m. the heat was turned off and at 12:34 p.m. the body of the subject was dry. When he left the bag at 12:45 p.m., he weighed 68.78 kgm. Between periods 2 and 3 and after period 4 of the respiration experiment skin-temperature measurements were taken.

Experiment VII (March 20, 1924). Mr. D. came without breakfast and lay down at 9:15 a.m. Nude weight 83.0 kgm. Mouth temperature, 36.0°C. Blood pressure, 126 mm. and 76 mm. Room temperature about 14°C. Pulse rate at 9:30 a.m. was 80 per minute. Respiration experiments were made at 9:45 a.m., 10:05 a.m., 10:30 a.m. and 10:52 a.m. At 11:10 a.m., after urinating, the weight was 82.77 kgm. He entered the bag and hot air was turned on at 11:17 a.m. The subject reported that the air felt much cooler than during his first experiment, which was on March 18. No metabolism experiments were made on this day after the subject entered the bag, as much time was occupied in testing the temperature of the air, which was subsequently found to be much lower than expected, due to defective connections with the heating resistances. Skin-temperature measurements were made at 11:03 a.m. and 12:20 p.m.

Experiment VIII (April 10, 1924). The subject, Dr. D. B., arrived at the laboratory at 9:10 a.m., after eating at 7:45 a.m. a non-stimulating breakfast consisting of 1 cup of caffeine-free coffee, 60 grams potato chips, and 30 cc. of cream. He lay down at 9:35 a.m. Respiration experiments were made at 10:14 a.m., 10:35 a.m. and 10:53 a.m.,[2] each of 6 minutes' duration. At 11:23 a.m. he entered the bag and the hot air was turned on immediately. His weight just before entering the bag was 75.00 kgm. The pulse rate was taken at intervals throughout the entire morning, usually by the sub-

[2] In the third experiment subject felt a little worried, as the experiment was made by a different operator and there was a little difficulty in the technique.

ject. The mouth temperature was taken frequently and the rectal temperature occasionally.[3] Respiration experiments were made at 12:06 p.m. and 12:29 p.m. The last experiment could continue but four minutes because "the high humidity caused by sweat collecting inside the bag, and high temperature of intake (94°C.) caused intense pain in the feet." The subject left the bag at 12:38 p.m., weighing 74.31 kgm.

DISCUSSION OF RESULTS. *Body weight.* One of the most pronounced results of a long sojourn in very dry air is a loss of weight. This is noted in table 2 in all of the experiments that we made. All of these individuals without exception had a very pronounced, visible perspiration during the experiment. Undoubtedly there was some increase in the intensity of loss of water both from the lungs and from the skin. The relatively small increase in metabolism would in part account for the increased loss

TABLE 2

Loss in body-weight during hot-air bath

SUBJECT AND DATE (1924)	TIME IN HOT AIR	INITIAL BODY WEIGHT	LOSS IN WEIGHT	
			Total	Per hour
	minutes	*kgm.*	*grams*	*grams*
Mr. D.:				
March 18	115	83.02	610	318
Mr. E.:				
March 15	100	69.97	470	282
March 16	110	69.30	400	218
March 17	95	69.75	500	316
March 19	105	69.20	420	240
Dr. D. B.:				
April 10	75	75.00	690	552
Mrs. F.:				
March 17	60	105.36	660	660
Miss G..				
March 18	150	90.80	870	348

from the lungs, but there would be an increased loss from the skin particularly on account of the high temperature and desiccation of the air. We were therefore prepared for rather large losses in body weight.

As seen from table 2, the losses range from as low as 400 grams, in the case of the experiment of March 16 with Mr. E. to as high as 870 grams, in the case of March 18 with Miss G. On the other hand, it is to be noted that the length of sojourn in the bag varies and that the longest experiment in the bag was that with Miss G., which fact alone might account for this greater loss of weight. The loss per hour, as shown in the last column of the table, has a wide range, from 218 grams on March 16 with Mr. E., to

[3] Rectal temperature at 9:35 a.m. was 37.2°, at 11:15 a.m. 37.1°, and at 12:42 p.m. 37.4°C.

as high as 660 grams with Mrs. F. The size of the individual should also be considered, and we notice that the loss per hour in general is larger, the larger the individual, the highest loss being 660 grams with Mrs. F., who weighed 105.4 kgm.

In computing these losses, it has been impossible to secure the exact time element, for the weights were taken just prior to the subject's entering the bag, and at the end of the experiment there was usually a short period of about 15 minutes for cooling off the subject with unheated air, before he came out of the bag and was weighed. It is quite evident, therefore, that the actual total loss due to the sojourn in the *hot air* was in every case somewhat less than that here indicated while the loss per hour, on the other hand, was really somewhat greater. In general the picture is that of a loss of from 220 to 660 grams per hour, the greater loss in weight being found with the larger individual. The length of time of exposure also probably plays some rôle.

The normal loss of individuals, even when nude in room air, would be not far from 40 to 50 grams per hour. Hence the exposure to this hot air has increased the loss from about 5 to 13 times, due almost exclusively to the increased amount of visible perspiration rapidly removed by the hot, dry air.

It has been common custom, in interpreting the results of many hot-air bath treatments, to consider that this loss is, in part at least, due to a loss of flesh or tissue, and in a reduction cure a loss, such as was displayed by Mrs. F., of 660 grams per hour might be considered of significance. As a matter of fact, the evidence in these experiments, so far as any reduction of tissue is concerned, is only partial inasmuch as thus far we have dealt only with the body weight. Later inspection of the actual changes in metabolism (see page 446) shows but minor increases in the oxidation of body tissue following exposure to hot air.

Respiration rate. With the three subjects (Mr. D., Mr. E. and Dr. D. B.) with whom respiration experiments were made, the respiration-rate was graphically written on the kymograph. The results are given in table 3. With Mr. D. there was a pronounced increase from an average of 14.3 respirations per minute before the exposure to hot air to approximately 20 after exposure, the rate remaining essentially constant throughout the entire sojourn in the bag. With Mr. E. the initial rates were somewhat lower, averaging not far from 12; during the first half-hour in the bag they tended to increase but slightly, and thereafter to resume the original rate of 12 or thereabouts. With Dr. D. B. the initial rate of 11 rose after a 30-minute exposure to 13.5 and fell again after the second half hour to 12.2.

Aside from the rather marked increase in the case of Mr. D. the effect of the hot-air bath upon the respiration-rate was not appreciable.

Pulse rate. With all subjects the pulse rate was taken before entering

the bag and in three cases during the sojourn in the bag. The data are recorded in table 4. Those for Mr. D. and Mr. E. are of significance only in showing the average pulse rate prior to the experiment. With Mrs. F., Miss G. and Dr. D. B. pulse rates were taken after entering the bag, when striking increases were noted in the case of Mrs. F. and Miss G. Thus, Mrs. F. before entering the bag had a pulse rate of 92, and after entering the bag an average pulse rate of 115 or a rise of 23 beats. With Miss G. the pulse rate rose from 78 to 112, i.e., 34 beats. Both of these women were large, Mrs. F. being very fat, and Miss G. fat and muscular. Accordingly it must be concluded that the influence of the hot-air temperature on these subjects was to increase the pulse rate materially. In the case of these two women subjects the pulse was counted by one of us at the wrist through a slit in the side of the oilskin bag. In the case of Dr. D. B., counts were made by himself inside the bag. In this case, prior to his entering the bag seven counts averaged 58 per minute. After he entered the bag, the pulse rate gradually increased fairly regularly, reaching finally a rate of 74 beats.

In all cases where the pulse rate was taken both before and after the subject entered the bag, a pronounced influence of the hot air in the form of an increase in the pulse rate was noted, the greatest increase being in the case of the two large, fat women subjects.

Mouth temperature. We have no data in which the subject's mouth temperature was measured both before and after entering the bag, save in the case of Dr. D. B. In the case of Mrs. F., after 15 minutes' exposure to the hot-air current, her temperature was but 36.9°C. It is unfortunate that later observations were not taken. Dr. D. B.'s observations are very accurate. The mouth temperatures prior to entering the bag averaged 36.4°C. The tendency then is for the mouth temperature to increase as the sojourn in the bag continues, until finally, after 75 minutes inside the bag, the temperature has risen to the slightly significant figure of 37.2°C.

Temperature and humidity conditions inside the bag. Air from the machine entered the bag near the right foot and passed out of the bag chiefly through a 5 cm. circular opening above the left shoulder, but also in part through leakage about the neck. The heat in the air is dissipated in two ways: first, through the walls of the bag, even if covered with a blanket; second, in vaporization of the large amounts of water poured out by the sweat glands on the skin of the subject. The temperature of the air at the entering point was usually somewhat over 80°C. It usually was discharged near the neck of the subject at not far from a temperature of 45°C. Thus a pronounced temperature gradient in the bag is indicated. Evidence regarding the nature of the temperature gradient was obtained, *a*, by placing a thermometer bulb between the bag and the covering blanket, which showed a pronounced decrease in temperature as the distance from the air-intake increased; *b*, by measuring the temperature of the air at

TABLE 3

Respiration-rate before and during hot-air bath

Average values per minute

SUBJECT AND DATE (1924)	BEFORE HOT-AIR BATH	IN HOT-AIR BATH		
		0–30 minutes	30–60 minutes	60–90 minutes
Mr. D.:				
March 18	14.3	20.0	19.9	19.7
Mr. E.:				
March 16	11.4	13.5	11.7	12.2*
March 17	11.9	13.2	12.4	12.8
March 19	12.5	—	12.2	11.5
Dr. D. B.:				
April 10	11.0	—	13.5	12.2

* Includes a rate of 12.2 noted after 90 minutes.

TABLE 4

Pulse rate per minute and mouth temperature before and during hot-air bath

SUBJECT AND DATE (1924)	PULSE RATE			MOUTH TEMPERATURE		
	Before hot-air bath (average)	In hot-air bath		Before hot-air bath	In hot-air bath	
		Time	Rate		Time	Rate
		minutes		°C.	*minutes*	°C.
Mr. D.:						
March 18	61	—	—	—	—	—
March 20	79	—	—	36.0	—	—
Mr. E.:						
March 16	50	—	—	—	—	—
March 17	54	—	—	—	—	—
March 19	55	—	—	36.1	—	—
Mrs. F.:						
March 17	92	22	116	—	15	36.9
		37	112			
		50	116			
Miss G.:						
March 18	78	73	112	36.8	—	—
Dr. D. B.:						
April 10	58*	7	54	36.4	16	36.6
		13	58		31	37.0
		24	62		51	37.1
		36	64		75†	37.2†
		51	74			
		62	68			
		72	74			

* Average of 7 values as follows: 64, 60, 58, 58, 56, 54, and 55.

† Taken 4 minutes after leaving bag.

various parts inside of the bag, particularly near the intake; *c*, by measuring the temperature of an envelope of air about 4 cm. from the skin. These air temperatures inside the bag were made with a thermo-electric junction and it was clear that there was a pronounced gradient in the bag between the points of exit and entrance, with a relatively cooler pocket of air over the right shoulder, *i.e.*, in a corner of the bag where the air circulation would not be active.

In the more exact study of the temperature conditions of the air in contact with the body the thermo-electric junction was held 4 cm. from the body, exactly over well-defined points subsequently used as landmarks in studying the surface temperature. This study was delayed until our last experimental day, when unfortunately the heating system failed and the hot air entering the bag was at a much lower temperature than usual. On this day, March 20, Mr. D. first placed the junction in contact with the skin at the several somewhat arbitrarily numbered points,[4] and the skin temperatures recorded in table 6 and designated "1 hour in hot air" were noted. Immediately thereafter he held the unprotected junction in the air, 4 cm. above each of the points recorded below. Locations 51 to 56 are along a median line from the suprasternal notch to the pubes, position 3 is on the left thigh, and position 8 is on the top of the right foot.

POSITION	AIR TEMPERATURE
	°C.
51	36.6
52	36.4
53	36.7
54	36.0
55	33.4
56	35.3
3	39.0
8	45.5

By reference to the values in tables 6 to 8 it can be seen that in general at the lower part of the legs the temperature was 4, 5 or even 6 degrees higher than on the skin itself. In the case of position 8 the difference is slightly over 8°C., but it should be noted that position 8 is very close to the entrance of the incoming air. From this last set of figures it can be seen that for the most part the subject was bathed in an air temperature about the legs very much higher than the skin temperature, but the upper part of the body was in an air temperature really not much higher than that of the skin temperature itself. Owing to the unfortunate breakdown in the

[4] A diagram of the positions and a key to the numbers have already been published by one of us (Benedict, 1925).

heating system, undoubtedly the absolute temperature at the various parts of the bag and around the body was somewhat lower, possibly 10° or 12°C., than on most of the other days, but in all probability the gradient was not much altered.

It would appear, therefore, that the loss of heat to the cool room through the bag (in spite of a blanket covering), the vaporization of water on the skin of the subject, and the absorption of heat by the relatively cool peripheral tissues, all combined to lower the temperature of the ambient air very rapidly, and it was only at the feet that unusually high temperatures of the air were maintained.

The humidity of the air leaving the bag depended naturally upon several conditions, chiefly the temperature of the air entering the bag, and particularly the length of sojourn of the subject inside the bag. Actual determinations of the water vapor per liter of air entering the bag just after the experiment on Dr. D. B. showed about 33 per cent humidity at 20°C. When the air is heated to 80° the humidity will be not far from 2 per cent.

On the whole we are not satisfied with our study of the humidity conditions inside the bag and relied for the most part upon the subjective impressions of the subject. It is to be noted that in the very last experiment Dr. D. B. was sweating profusely above the thighs. His feet, exposed to a blast of air at 94°C. with a humidity below 2 per cent, were dry but almost unbearably hot.

Rectal and vaginal temperatures as influenced by a sojourn in a hot-air current. While the mouth temperatures were hardly of significance and showed little, if any, result of the prolonged sojourn in the hot air, it was deemed desirable to make a more careful study of the rectal or vaginal temperatures and as nearly as possible to make the observations continuous. We accomplished this by means of a thermo-junction, and we have observations prior to and during the sojourn in the bag with Mr. D., Mr. E. and Miss G. With Dr. D. B. we have clinical thermometer records taken prior to entering the bag and one at the conclusion of the sojourn in the bag. All of the pertinent data are recorded in table 5. In this table the rectal temperatures are recorded prior to entering the bag and at intervals of 5 minutes after entering the hot-air current, to note any influence of the prolonged sojourn. With Mr. D. the initial rectal temperature was 36.8°C. on March 18 and 36.5°C. on March 20. On the first day there was practically no influence noted with the sojourn in the hot air, for after 60 minutes in the bag the temperature was slightly lower than it was at the start. On March 20 there was an insignificant rise throughout the day.

With Mr. E., whose sojourn in the bag was somewhat longer, the rectal temperature on March 16 rose from 36.7°C. to a maximum of 37.3°C.,

hardly a significant rise. On March 17, although no records were made prior to entering the bag, the first record, five minutes after the exposure began, was 37.0°C. with a rise to 37.4°C. at the end of an hour and a half, i.e., no appreciable effect. A similar experience was noted on March 19, when the temperature rose from 36.8° to 37.4°C.

With the third male subject, Dr. D. B., the temperatures prior to entering the bag were 37.2° and 37.1°C., respectively, and increased to but 37.4°C. after one hour and a half, i.e., an insignificant increase.

TABLE 5

Rectal and vaginal temperatures before and during hot-air bath

MINUTES IN HOT-AIR	MR. D.		MR. E.			MISS G.
	March 18	March 20	March 16	March 17	March 19	March 18
	°C.	°C.	°C.	°C.	°C.	°C.
Before	36.8	36.5	36.7	—	—	—
5	36.8	—	36.7	37.0	36.8	37.3
10	36.2	—	36.7	36.8	36.9	37.4
15	36.8	—	36.8	37.0	37.0	37.6
20	—	—	36.9	—	37.0	37.7
25	36.8	36.6	36.8	—	—	37.8
30	—	36.6	37.0	—	36.1	—
35	37.0	36.8	37.0	37.1	37.2	—
40	36.9	36.7	37.1	37.1	37.3	—
45	—	36.8	37.1	—	37.2	38.0
50	36.6	—	37.1	37.2	37.3	38.1
55	—	36.8	—	37.3	37.2	38.2
60	—	—	37.2	—	37.4	38.0
65	—	—	—	—	—	—
70	—	—	37.2	—	—	38.3
75	—	—	37.3	37.4	—	—
80	37.1	—	37.3	37.4	—	—
85	37.0	—	—	—	—	—
90	—	—	37.1	37.4	—	—

On the contrary, with the large, fat, powerful woman, Miss G., the vaginal temperatures changed pronouncedly from 37.3° to 38.3°C. in somewhat over an hour. Under these conditions it is very much to be regretted that we could not have taken the rectal or vaginal temperatures of the other fat, female patient.

During most of the periods of the day when these subjects were inside of the chamber or bag, there would be normally an increase in the rectal temperature due to the diurnal rhythm. This might wholly explain the slight increases noted in the case of Mr. D. and Mr. E., and indeed that of Dr. D. B. On the other hand, no such explanation can be accorded to the distinct rise in temperature noted with Miss G., that is, a rise of 1°C. in one

hour and 10 minutes. The fact that neither of the three men showed an appreciable effect of the hot-air environment upon the deep trunk temperature, and that the fat woman, Miss G., did show a pronounced effect, may perhaps be explained by the disturbance of heat loss due to her rather large protective layer of fat.

The temperature gradient in the body. Under ordinary conditions there is a steep and fairly sharp temperature gradient between the vagina or rectum and the skin. A study is readily made of this gradient by means of a thermo-electric couple inserted deep in the rectum or vagina and gradually withdrawn, the fall in temperature as it approaches the surface being thus noted. With one of our subjects, Miss G., such measurements were made. Prior to entering the bag the junction inserted into the vagina indicated a deep temperature of 37.3°C. Upon withdrawing this to within 2 cm. of the surface, the temperature had fallen to 36.96°C. After a sojourn inside the apparatus, the thermal gradient at 3:50 p.m., i.e., an hour and 20 minutes after entering the bag, completely disappeared, for the temperature deep in the vagina was 38.3°C., while at the surface it was 38.2°C.

Under these conditions it can be seen that practically all the cells in the body were at a temperature essentially that of the rectum or vagina, and that the peripheral mass of tissue which, with an ordinary person (even when well covered with blankets in bed) normally may be two, three or more degrees below the temperature deep in the body trunk, now has the same temperature.

Skin temperature. By means of the simple thermo-electric technique, a temperature survey of the nude skin could rapidly be made and comprised a part of our study. (See tables 6 to 8.) Whatever slight influence could be noted in the rectal temperature as a result of the prolonged exposure to hot air, material changes in skin temperature were to be expected in spite of the fact that there would be an increased vaporization of water, tending in turn to lower the skin temperature. These skin temperatures were first taken with the subject lying covered in bed, and may be considered as normal skin-temperature measurements. They preceded all the experiments made in the bag, and we have 2 days with Mr. D., 3 days with Mr. E., 1 with Mrs. F., and 1 with Miss G. The arbitrary numbering of the positions, which follows long usage in the Nutrition Laboratory, may be summarized by stating that the first two are on the face, position 26 on the forehead and 101 on the left cheek; the next group, positions 51 to 14, inclusive, are on the trunk, most of them in the median line; the next group of 3 are on the arm; and the last long group on the thighs, legs and feet, positions 24 and 25 being on the bottom and positions 7 and 8 on the top of the left and right foot, respectively.

Considering, first, the normal measurements taken in bed prior to the

experiment, one sees that in general the highest temperatures are noted on the trunk and the lowest, as is to be expected, on the extremities, particularly the feet. Certain of these data are being reported elsewhere in a general article on skin-temperature measurements (Benedict, 1925). On March 17 with Mr. E. the experimental conditions differed in that a

TABLE 6

Skin temperatures before and during hot-air bath, Mr. D.

POSITION	MARCH 18, 1924			MARCH 20, 1924	
	Normal*	In hot-air bath		Normal*	In hot-air bath
		1 hour	1½ hrs.		1 hour
	°C.	°C.	°C.	°C.	°C.
26	32.2	—	—	31.1	—
101	32.1	—	—	30.9	—
51	34.3	36.3	37.6	33.3	36.0
52	34.1	35.8	36.2	33.0	35.8
53	34.6	35.7	35.3	34.0	35.7
54	34.2	34.7	35.2	34.2	34.6
55	33.9	35.1	36.0	34.2	35.0
56	33.8	35.3	36.0	34.2	34.2
15	—	—	35.0	34.5	35.5
14	34.6	34.3	34.9	34.3	34.7
23	32.4	36.3	36.2	32.5	—
11a	33.6	36.8	36.7	33.6	—
18a	33.9	35.8	35.9	33.5	—
3	33.2	35.5	35.5	32.8	35.7
2	33.8	35.4	35.3	33.0	35.4
4	—	36.1	36.0	31.6	—
10	—	36.4	36.1	31.9	—
1	33.2	35.6	35.3	32.0	—
9	32.9	36.2	36.5	32.4	—
7	—	—	38.0	28.6	—
24	—	—	36.6	27.2	—
8	32.1	36.7	35.8	29.8	37.3
25	30.7	36.1	36.2	29.0	37.6

* Subject had been lying in bed, well covered, for 2 hours; average room temperature 17°C.

hot-water bottle (the temperature of the hot-water bottle was unfortunately not recorded) was placed at the feet, by which apparently the temperature throughout the entire body, save on the two exposed places, the forehead and cheek, was equalized. Miss G. showed very high temperatures of considerable uniformity throughout the body, without a pronounced falling off at the feet. It is barely possible that the protection of the shoes

and stockings, which had been worn all the morning, kept her feet at a high temperature, so that the temperature did not fall off. Reference to tables 6 to 8 shows that under the so-called normal conditions (with the two exceptions above noted) the temperatures of the feet are much colder than the rest of the body, but as the entrance of the hot air from the machine was

TABLE 7

Skin temperatures before and during hot-air bath, Mr. E.

POSITION	MARCH 16, 1924		MARCH 17, 1924			MARCH 19, 1924	
	Normal*	In hot-air bath	Normal*	In hot-air bath		Normal*	In hot-air bath
		1½ hours		1 hour	1½ hours		1 hour
	°C.	°C.	°C.	°C.	°C.	°C.	°C.
26	32.6	—	32.8	—	—	32.4	—
101	33.3	—	33.7	—	—	32.6	—
51	34.2	36.8	34.4	36.8	35.6	34.1	35.9
52	34.2	—	34.9	36.4	35.2	34.6	35.1
53	33.8	36.0	34.8	36.1	34.9	34.6	35.0
54	33.6	34.8	34.6	36.1	34.6	34.3	35.3
55	33.3	34.2	34.1	35.5	34.9	33.9	34.8
56	33.7	34.1	33.9	35.0	34.6	34.1	35.6
15	33.7	—	—	—	—	34.5	—
14	33.5	—	34.4	37.3	37.5	34.2	35.5
23	32.3	—	34.0	—	—	32.9	34.2
11a	32.0	—	33.8	—	—	33.4	34.9
18a	33.1	—	34.4	—	—	33.6	34.5
3	32.8	35.0	33.5	35.9	35.2	32.5	35.4
2	32.3	34.8	33.8	35.3	35.2	32.4	34.8
4	—	35.9	35.1	36.6	36.3	31.4	36.2
10	—	35.9	35.2	35.9	35.6	32.4	36.2
1	32.8	35.0	34.3	36.7	34.8	33.0	37.0
9	32.0	35.0	33.3	35.7	35.2	32.6	55.9
7	—	—	34.6	36.5	36.1	29.4	37.4
24	—	35.9	34.1	36.1	37.3	28.1	37.9
8	30.3	—	34.2	35.8	36.0	28.5	36.9
25	26.3	35.7	34.2	36.8	36.8	27.9	36.9

* Subject had been lying in bed, well covered, for 2 hours; average room temperature 17°C.

near the right foot, the higher temperature at the feet when inside the bag might be expected and would account, in part at least, for the extraordinarily high temperatures noted occasionally, as in the case of Miss G., when a temperature as high as 41.5°C. was noted on position 10, one-half hour after she entered the bag.

It is clear, therefore, from a study of tables 6 to 8 that the result of bathing the body in hot air is to secure throughout the entire skin surface a fairly uniform temperature which is, on the average, one or more degrees higher, even at the normally warm parts of the trunk, than is commonly

TABLE 8

Skin temperatures before and during hot-air bath, Mrs. F. and Miss G.

POSITION	MRS. F.				MISS G.		
	MARCH 17, 1924				MARCH 18, 1924		
	Normal*	In hot-air bath			Normal*	In hot-air bath	
		½ hour	½ hour	¾ hour		½ hour	1 hour
	°C.	°C.	°C.	°C.	°C.	°C.	°C.
26	32.3	—	—	—	32.2	—	—
101	32.3	—	—	—	33.0	—	—
51	35.2	36.6	37.1	38.1	35.4	37.6	36.3
52	34.5	36.2	36.9	37.3	34.6	36.8	36.2
53	33.9	35.4	36.1	36.6	34.9	36.1	35.9
54	34.2	36.4	36.8	37.3	35.3	36.1	35.8
55	33.0	38.6	38.0	38.3	34.5	36.6	36.0
56	34.9	38.6	38.8	39.6	35.4	36.4	36.1
15	35.0	36.2	36.5	37.4	35.4	35.7	35.0
14	35.2	36.0	36.4	37.0	35.4	36.0	36.0
23	—	35.5	36.3	37.3	31.7	37.3	36.9
11a	34.6	35.8	36.3	37.3	34.1	37.3	36.7
18a	33.3	36.1	35.2	36.3	34.3	37.1	36.7
3	32.9	37.9	38.7	39.9	34.1	37.9	37.2
2	32.7	37.5	38.1	38.3	33.1	38.0	36.8
4	31.1	39.6	39.6	40.5	33.4	41.0	40.0
10	30.7	35.6	37.2	38.7	34.1	41.5	39.6
1	32.4	—	—	—	34.6	39.4	—
9	31.4	—	—	—	33.8	38.1	39.0
7	29.0	36.9	36.8	37.9	33.2	39.6	39.6
24	30.1	37.9	39.0	40.3	32.6	39.6	39.6
8	30.6	—	—	—	33.5	38.6	38.5
25	28.5	—	—	—	34.0	38.5	39.6

* Subject had been lying in bed, well covered, for 2 hours; average room temperature 17°C.

the case. Indeed, frequently these temperatures are several degrees higher, notably in the case of Mrs. F. on March 14, where the temperature at position 55 showed an increase of 5.5°C. in the first quarter of an hour. It has been noted, however, that in no case, save perhaps in the final period with Mrs. F., was the skin temperature persistently higher than

the normal rectal temperature. Unfortunately her rectal and vaginal temperatures were not taken, but it is safe to predict that they were considerably above normal.

The large losses in body weight already noted, due almost entirely to loss in vaporization of water, clearly account, therefore, for the failure of the skin temperature to rise perceptibly above the rectal temperature, owing to the cooling effect of the vaporization of water. It would thus appear that the body under these conditions acted much as a wet-bulb thermometer.

Gaseous metabolism. According to the impressions of these subjects and others who had submitted themselves to the hot-air blast, the sensations were very much like those noted by subjects immersed in a neutral bath (Benedict and Benedict, 1924), i.e., for the most part very agreeable. Nevertheless, from the studies on the rectal and particularly the skin temperatures, it is obvious that placing a nude human subject in an environmental temperature in which hot air is blowing over him must result in a profound disturbance of heat loss and a change in skin temperature. Although these temperature studies indicate that except in the case of the fat woman there was no pronounced change in trunk temperature, hot air treatment does definitely raise the peripheral temperature to a point not far from that of the trunk. It is obvious that under these conditions we must consider the possible effect of a temperature increase upon cell activity, particularly in the peripheral tissues. On the other hand, the extensive vaporization of water from the surface of the body results in a pronounced cooling effect, which in part offsets the excessive heat in the air. These two factors affect considerably the temperature of the subcutaneous tissue and somewhat the temperature deep in the trunk.

The effect of these increases in the temperature of these tissues upon cell activity, as indicated by the total metabolism, could not be predicted. In one of the bath experiments where the temperature of the bath water was somewhat above that of the rectal temperature, an appreciable increase in basal metabolism was noted. The differences, however, in the environmental temperature of a warm water bath as compared to that of a stream of hot air are very pronounced. In the neutral water bath all loss of heat by vaporization of water was precluded. In the case of heated air the total loss of heat must have been by means of the vaporization of water, since the environmental temperature was practically always at or above the tissue temperature. These fundamental differences in heat loss demand, we believe, a study of the influence of such thermal mediums upon the gaseous metabolism.

Fortunately gaseous metabolism measurements could easily be made and graphically recorded. The data are given in table 9.

Most of the measurements of the gaseous metabolism were made with

Mr. E. His oxygen consumption averaged on the first day prior to entering the hot-air chamber (March 16) 219 cc.; on March 17, 220 cc.; and on March 19, 219 cc. After he entered the hot air, the course of the metabolism was not identically the same on the three days. While on the first two days there was a reasonably consistent increase (as is seen in table 9), on March 19 there was a pronounced increase and higher values for both periods. Expressed in terms of percentage, there was an increase due to the hot air of from 2 to 10 per cent on March 16 and from 6 to 14 per cent on March 17; on March 19 there was a maximum increase of 33 per cent in the first period and an increase of 17 per cent in the second period.

With Mr. D. measurements were made on March 18 and 20, prior to his entering the bag. On only one day, March 18, were measurements made after exposure to hot air. On March 18 the basal metabolism steadily declined during the 3 periods before he entered the bag, with a minimum of 253 cc. of oxygen. On March 20 the 3 periods show, if anything, a

TABLE 9

Oxygen consumption (in cubic centimeters per minute) before and during hot-air bath

SUBJECT AND DATE	BASAL			IN HOT-AIR BATH			
Mr. D.:							
March 18	288	274	253	277	283	283	
March 20	265	263	274	—	—	—	
Mr. E:							
March 16	222	217	217	222	229	242	235
March 17	219	222		250	237	232	
March 19	223	216		292	255		
Dr. D. B.:							
April 10	248	248	259	255	269		

slight tendency to increase, with a minimum of 263 cc. After Mr. D. entered the bag on March 18 the oxygen consumption increased to 277 and 283 cc., an increase of about 8 per cent.

Finally, with the experiment of Dr. D. B., we have three periods prior to his entering the bag, which give an average oxygen consumption of 251 cc. The metabolism was also measured about one-half hour after the subject entered the bag, and again about 50 minutes after entering the bag. In period 4 the oxygen consumption was 255 cc. and in period 5 it was 269 cc. There was an increased metabolism over basal of but 2 per cent in the fourth period and 7 per cent in the fifth.

Since the loss of heat due to radiation is entirely eliminated, at least in large part (although it may possibly be that the temperature of the air about the upper chest and shoulders was not always appreciably above that of the skin temperature), we have to deal with a loss of heat from the body due entirely to loss of water. On the other hand, the increased

temperature of the peripheral tissue must have stimulated cell activity much as in the case of fever, and hence resulted in an increased metabolism.[5] The resulting effect is, on the whole, a somewhat increased metabolism, the increase amounting to approximately 5 to 10 per cent after a sojourn of from 1 to 1½ hours in this hot-air bath.

The possibility of loss of heat in large amounts from the human body by vaporization of water, the possibility of actual absorption of heat from the hot-air environment, and an increased metabolism in certain instances—all physiological factors which must approximate at least those experienced in the tropics—would seem to make a study of the metabolism and heat regulation of people living in the tropics of unusual physiological significance.

SUMMARY

The effect of exposure of the nude human body to a current of hot air having a temperature of approximately 80° to 85°C. (176° to 185°F.) was studied with 3 men and 2 women, with the following results.

1. There was a loss in body-weight of from 220 to 660 grams per hour, this loss in general being larger the larger the individual. The length of exposure to the hot air also undoubtedly played some rôle. The normal loss of a nude human in room air would be about 40 to 50 grams per hour. Hence the loss due to exposure to hot air would be 5 to 13 times greater. This increased loss is due almost exclusively to the increase in visible perspiration. Measurements of the oxygen consumption show that it probably represents only a minor increase in the oxidation of body tissue.

2. The respiration rates were determined in the case of the 3 men. With one the hot air caused a marked increase in the respiration rate; with the other two it had no appreciable effect.

3. The pulse rate was definitely increased by the hot air.

4. Records of the mouth temperature before and after the sojourn in the hot-air bath were obtained with only one subject. In this case the mouth temperature tended to increase as the sojourn in the hot air continued, reaching a value of 37.2°C. at the end of 75 minutes.

5. There was a marked temperature gradient in the air entering and leaving the hot-air machine. Thus, the temperature of the air at the point of entrance was usually somewhat over 80°C. and at the point of exit not far from 45°C. The temperature of the air about the legs of the subject was therefore very much higher than his skin temperature, but the temperature of the air around the upper part of the body was not much higher than that of the skin temperature itself. The study of the humidity

[5] It has been shown by one of us (Du Bois, 1924) that each degree rise in temperature (Centigrade) corresponds to an increase of about 13 per cent in the metabolism.

of the air was unsatisfactory and the subjective impressions of the subjects had to be relied upon.

6. The hot-air bath had no appreciable effect upon the rectal temperatures of the 3 men, but it caused a pronounced increase in the vaginal temperature of the one rather stout woman subject with whom this measurement was obtained. The diurnal rhythm in the rectal temperature might explain the slight increase noted in the case of the 3 men, but does not explain the distinct increase of 1 degree in 70 minutes noted with the woman. The explanation in the case of the woman may perhaps be that the rather large protective layer of fat disturbed the loss of heat.

7. The temperature gradient ordinarily existing between the vaginal or rectal temperature and the skin temperature completely disappeared as a result of the hot-air bath in the case of the woman subject with whom vaginal temperatures were obtained.

8. The skin temperature became fairly uniform over the whole body as a result of the hot-air bath and on the average was one or more degrees higher than the skin temperature under ordinary conditions. In no case, however, was the skin temperature during the hot-air bath persistently higher than the normal rectal temperature, owing to the cooling effect of the vaporization of water from the skin.

9. Respiration experiments with the 3 men indicated a somewhat increased oxygen consumption as a result of the hot-air bath, this increase amounting to from 5 to 10 per cent after a sojourn of from 1 to $1\frac{1}{2}$ hours in the hot air.

10. The results of these hot-air experiments lead to the suggestion that a study of the metabolism and heat regulation of people living in the tropics would be of unusual physiological significance.

BIBLIOGRAPHY

BENEDICT, F. G. 1925. Asher-Spiro's Ergebnisse der Physiologie, Supplement Band, (Festschrift für Leon Asher).

BENEDICT, C. G. AND F. G. BENEDICT. 1924. Proc. Nat. Acad. Sci., x, 495; Bulletin Soc. Sci. d'Hygiène Alimen., xii, 541.

BENEDICT, F. G. AND W. E. COLLINS. 1920. Boston Med. Surg. Journ., clxxxiii, 449.

BENEDICT, F. G., W. R. MILES AND A. JOHNSON. 1919. Proc. Nat. Acad. Sci., v, 218.

DU BOIS, E. F. 1924. Basal metabolism in health and disease. Philadelphia, 330.

PEMBREY, M. S. 1898. Schaefer's Text-book of physiology. Edinburgh, London and New York, i, 814.

ROTH, P. 1922. Boston Med. Surg. Journ., clxxxvi, 457.

THE BASAL METABOLISM OF SOME ORIENTALS

GRACE MacLEOD, ELIZABETH E. CROFTS AND FRANCIS G. BENEDICT

From the Department of Nutrition, Teachers College, Columbia University, New York; the Department of Physiology, Mount Holyoke College, South Hadley, Massachusetts; and the Nutrition Laboratory of the Carnegie Institution of Washington, Boston, Massachusetts

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At that period in the history of nutrition investigations when unusual stress was laid upon the protein intake, particularly for the performance of muscular work, the large amount of work performed by the Japanese jinrikisha men, accompanied by their low nitrogen and presumably low caloric intake, was frequently cited. Thus, the general conception has long prevailed that the Chinese and Japanese (and these two races alone are here considered) have lower food needs than Americans and Europeans. An estimate of the food requirements of the individual, even when based upon well-conducted dietary studies, is at best unreliable. It is impossible from such studies to deduce the probable basal metabolism, and it is only with the advent of modern metabolism technique that one can speak with surety regarding the basal metabolism of individuals. Thus far, however, there are extremely few published reports of the basal metabolism of Chinese and Japanese.

In connection with researches in progress in our several laboratories, certain observations upon Chinese and Japanese college women were secured. With these women some unusual phases of metabolic activity were noted, which challenged our attention when comparison was made with American college girls in the same environment. It has been our experience that in studying any group of 15 or more individuals (even though they are seemingly normal people, that is, in presumably good health) it is safe to predict that in such a group one or more subjects will be found with a variation in metabolism of 15 per cent or more above or below any of the commonly accepted normal standards. All individuals showing such deviations from normal standards should receive special study, with an attempt to find out why such extremes exist without any obvious pathological lesion.

Previous investigations with Orientals. The literature on the subject of the metabolism of Orientals is strikingly meager. We have been unable to find any metabolism measurements on normal Chinese and

Japanese women living under American conditions, save in the case of the extraordinarily well studied subject I of Prof. Katharine Blunt and Miss Dye (1). This subject (who was 21 years of age, with a height of 160 cm., a weight of 58.5 kgm., and a surface of area of 1.62 sq. m.) showed as an average of 26 observations between January 27 and March 5, inclusive, an average pulse rate of 62 beats per minute, and a total heat production per 24 hours of 1,239 calories, or 31.9 calories per square meter of body surface per hour. This is a metabolism about 14 per cent below the Aub and Du Bois prediction. It is, however, important to note that on at least one day this subject had a heat production of 28.9 calories per square meter of body surface per hour, and on another day, 5 days later, 29.7 calories. If one considers the average of these two values as representing more strictly the true basal metabolism, the deficit in metabolism becomes nearer 21 per cent.

Fragmentary observations bearing somewhat upon the basal metabolism of Orientals occasionally appear in the literature. Thus, Yano (2), in discussing the metabolism of Japanese infants, comments particularly upon the low energy metabolism of the Japanese infant and suggests that it may be a characteristic of the Japanese race.

A series of papers has been published by Cadbury (3) on the blood pressure and pulse rate of Cantonese students, and his measurements have been supplemented by a study of the blood pressure of Chinese in North China by Cruickshank (4). The general trend of these studies shows that these students have a low blood pressure, a low pulse rate and low alveolar air.

Foster (5) finds that the vital capacity of the healthy Chinese is considerably below the standard adopted by Americans, and specifically states that 75 young women from the Union Girls' School and from the Hunan-Yale Nursing School, ranging from 17 to 25 years of age, gave figures for vital capacity 30 per cent lower than the standard for American women.

Fleming (6), in making observations on Filipinos (men), reported that his 8 normal subjects all had a basal metabolism rate below the normal standard, the difference in two cases being greater than −13 per cent. The average deviation for the eight men was −5.3 per cent. These data are complicated, however, by the fact that, in the first place, the subjects were so-called "hospital normals," measurements on which we are quite averse to employing in discussing normal metabolism rates. In the second place, the measurements were made under conditions more closely approximating the tropical conditions, the influence of which is still under debate and is not to be considered by us in connection with our study of Orientals.

Much more in line with our findings, however, although unfortunately

as yet unaccompanied by experimental evidence, is the significant statement of H. G. Earle in a very remote publication published by the Hongkong University Medical Society in Hongkong (7). In discussing the effect of race and climate, Earle states:

> We hope to publish detailed results later, but from the 50 or so observations already made on the normal Chinese student, making all allowances for stature, weight, sex and age to which reference has been made, it would appear that the metabolic pace is set at a lower level than that of western subjects and the question arises how far this can be correlated with other differences between the eastern and western peoples.

Earle also asks three significant questions. Has the rush of western civilization produced a higher metabolic rate? Is the low metabolic rate of the Chinese a physiological expression of their more philosophical outlook on life? How far do climatic conditions, apart from race, affect the metabolic rate?

Observations and technique. It is important to note at the outset that we are not dealing here with the mooted question of the influence of temperature, humidity and dietetic habits of the tropics which has been so stoutly discussed, with varying opinions, by Eijkman (8), Knipping (9), Almeida (10) and others. Our studies were made upon Chinese and Japanese young women, doing collegiate work in two American colleges in New York City and South Hadley, Massachusetts, under essentially the same climatic conditions. A careful inspection of their dietary habits and general habits of life leads us to believe that they very closely resemble those of the normal woman student of the American college. Indeed, it is clear that, since coming from the Chinese and Japanese environment to that of the active American college, these young women have in part at least been infused with the rush of western civilization, for they are all characterized by having become intensely interested in their collegiate life, with anything but the supposedly lethargic action of mind and body of the Oriental. Furthermore, the climatic and dietetic conditions are now, and have been for some time in many of our cases, similar to those of American young women with whom they are compared.[1]

The experiments were all made in the winter months, i.e., between November and April. Observations on 7 Chinese and 2 Japanese women are reported, including the usual records for age, height, weight, sitting height, surface area and, in addition, the pelidisi. The physiological observations include the pulse rate, respiration rate and vital capacity. On three subjects the systolic and diastolic blood pressure was determined,

[1] The length of time since these women had left their Oriental homes was as follows: no. 1, 20 months; no. 2, 15 months; no. 3, 18 months; no. 4, 30 months; no. 5, 20 months; no. 6, 27 months; no. 7, 52 months; no. 8, 15 months; and no. 9, 27 months.

and with the same women the surface area was obtained from direct anatomical measurements, by employing the Du Bois linear formula (11).

A tested type of the Nutrition Laboratory respiration apparatus, provided with Sadd valves, was employed for the measurements of the oxygen consumption. In one laboratory the two apparatus used had actually been tested in the Nutrition Laboratory by means of alcohol-check experiments. In the other laboratory the portable respiration apparatus was subjected to a physiological test in that measurements were made on the same subject with two entirely distinct respiration apparatus, i.e., the portable respiration apparatus and the student respiration apparatus. This test gave closely agreeing results. With all the apparatus used careful tests for leaks were made by placing a weight upon the spirometer bell half-way through the experiment. Thus, if a leak was present, the change in rate of the fall of the spirometer bell as the experiment progressed was indicated immediately on the graphic chart. There were no leaks.

At this point it is important to emphasize the necessity for the strictest control of the accuracy of the various types of respiration apparatus now upon the American market, which are only too frequently put out by the manufacturer without any guarantee of accuracy. In many cases such apparatus can be actually tested by calipering the spirometer bell, particularly if the bell is of the cylindrical type; in other cases alcohol-check experiments can be made. Finally, in those laboratories having two totally different types of respiration apparatus the physiological test, as outlined above, may be made by measuring the metabolism of the same subject with two different forms of apparatus.

All our measurements were made in the post-absorptive condition, that is, at least 12 hours after the last meal, with avoidance of muscular activity and with due regard to the importance of psychic repose. It is perhaps unfortunate that in a study of this kind it was necessary to include two cases, no. 1 and no. 8, in which the metabolism was characterized by a rather striking variability from day to day. For normal physiological studies, therefore, neither of these women could be considered an ideal subject. In the general belief that it is much easier to secure muscular and nervous conditions where the metabolism will be *above* rather than below the so-called basal, we have reported for these two women values based upon at least two closely agreeing *minimum* periods, even if determined on two different days, provided they were not over a week apart.

DISCUSSION OF RESULTS. *Physical characteristics.* The main findings of this study are shown in abstract in table 1. In the first place, certain features of the physical characteristics of the subjects demand special consideration.

These young women were all college students, ranging in age from 21 to

29 years, the average age being 24 years. The well-known relatively small size of the Chinese and Japanese accounts immediately for the small average body-weight of 47.2 kgm., as compared with the average weight of 56.5 kgm. for the 103 American women studied by the Nutrition Laboratory (12) and the average weight of 57.2 kgm. for the 43 American women in the age group from 20 to 30 years studied at Teachers College. Similarly, the characteristic Oriental shortness of stature is shown here, the average height being but 155 cm., as compared with the average height

TABLE 1

Basal metabolism of 9 Oriental women

SUBJECT NUMBER	DATE	AGE	NUDE WEIGHT	HEIGHT	SITTING HEIGHT	PELIDISI	BODY SURFACE*	VITAL CAPACITY	VITAL CAPACITY DIVIDED BY		PULSE RATE	RESPIRATION RATE	O_2 PER MINUTE	HEAT PRODUCED PER 24 HOURS		HEAT PRODUCED PER SQUARE METER PER HOUR	
									Height	Body surface				Total	Deviation from Harris and Benedict standard	Total	Deviation from Aub and Du Bois standard
		years	*k.m.*	*cm.*	*cm.*		*sq. m.*	*liters*	*cc.*	*liters*			*cc.*	*calories*	*per cent*	*calories*	*per cent*
Chinese																	
1	Apr., '23	21	49.3	159	84.9	93	1.48	2.29	14.4	1.54	58	18	183	1,271	−3.9	35.8	−3.2
2	Dec., '24	25	54.5	162	88.2	93	1.57	2.23	13.8	1.42	58	14	173	1,202	−11.6	31.9	−13.8
3	Dec., '24	29	56.2	147	84.5	98	1.48	2.02	13.7	1.36	64	15	187	1,299	−2.3	36.6	−1.1
4	Feb., '25	22	44.5	156	86.6	88	1.39	2.04	13.1	1.47	59	16	162	1,126	−11.1	33.8	−8.6
5	Feb., '25	25	44.5	155	83.1	92	1.39	2.14	13.8	1.54	64	13	165	1,146	−8.4	34.4	−7.0
6	Dec., '24	21	45.6	151	78.2	99	1.43	2.00	13.2	1.40	63	14	154	1,070	−15.9	31.2	−15.7
7	Nov., '24	23	49.6	159	88.2	90	1.55	2.70	17.0	1.74	59	16	162	1,126	−14.4	30.3	−18.1
Japanese																	
8	Nov., '24	23	38.4	148	82.7	88	1.32	2.20	14.9	1.67	58	9	154	1,070	−9.9	33.8	−8.6
9	Nov., '22	28	42.2	155	83.3	90	1.35	2.28	14.7	1.69	54	12	146	1,014	−16.5	31.3	−15.4
Average		24	47.2	155	84.4	92	1.44	2.21	14.3	1.54	60	14	165	1,147	−10.4	33.2	−10.2

* Body surface determined from Du Bois height-weight chart, except in the case of Nos. 6, 7 and 8, when it was actually determined.

of 162 cm. for the Nutrition Laboratory women and of 163 cm. for the Teachers College group.

Owing to the significance that has been attributed to the sitting height, particularly in the use of the pelidisi as an index of the "state of nutrition," both measurements are recorded in table 1, but there are altogether too few recorded sitting heights of college women for comparison purposes. The average measured sitting height of these Orientals was 84.4 cm., while with 32 of the Teachers College group the measured sitting height was 87.2 cm. Pirquet has suggested that the sitting height bears a very

close relationship to the length and can be computed by adding 5 cm. to one-half the length in centimeters (13). This simple calculation has been carried out for these young women, and we find that the average value, as thus calculated, would be 82.3 cm. or 2.1 cm. less than that actually measured. With 32 of the Teachers College group the calculated sitting height was but 0.9 cm. less than the measured sitting height. In a number of instances the sitting heights of the Orientals were very long, indicating long bodies. Thus, for example, with subject 3 the measured sitting height was 84.5 cm. as compared with the computed value of 78.5 cm.; with subject 7 the measured sitting height was 88.2 cm. and the computed value is 84.6 cm.

Pelidisi. The importance of knowing the "state of nutrition" of subjects undergoing basal metabolism measurements has recently been emphasized (13). In this discussion it was pointed out that the ordinary method of indicating the state of nutrition by the terms "good" "moderate" or "bad" would no longer suffice, and that some more specific index would soon be necessary. It was proposed, in considering the metabolism of a large series of young girls (13), that the pelidisi suggested by Pirquet was worthy of trial. The pelidisi, as determined by Pirquet, represents the relationship between the weight and the sitting height. This relationship is calculated by dividing the cube root of ten times the weight in grams by the sitting height expressed in centimeters. The values obtained by this computation range usually somewhere between 85 and 115, but it is important to note that 100 does not represent normal, which is rather nearer 95. Not infrequently the sitting heights of Orientals indicate long trunks, and it is not impossible that this length may affect the interpretation of the pelidisi, and that the pelidisi index suggested by Pirquet, which is derived chiefly from measurements on white races, cannot be applied to Orientals any more than the standards of vital capacity can be thus applied. The pelidisi have been computed, however, in the case of our 9 subjects, and we find that they range from 88 in the case of subjects 4 and 8 to 99 in the case of subject 6. In general these values are a little low rather than high, being on the average 92 as compared with the average pelidisi of 95 noted in the case of the Teachers College group 20 to 30 years old. This latter value of 95 is the pelidisi usually considered as representing an average normal state of nutrition. Very little significance can be attached to the picture shown by the pelidisi of these Orientals, however, other than that our subjects were neither pronouncedly over- nor under-nourished.

Surface area. The surface areas of these women were for the most part taken from the Du Bois height-weight chart (11), using the nude weight and height as recorded in table 1. They are on the average 1.44 sq. m., which is in conformity with the relatively small body-weight.

Having in mind that the vital capacity standards do not apply to Orientals and that possibly the pelidisi may not apply, we considered it desirable, in certain cases at least, to make actual measurements of the body surface from the anatomical measurements of the subjects, in accordance with the Du Bois linear formula (11). In the case of subjects 6, 7 and 8, therefore, such actual measurements were made and the measured area, not the computed area, is recorded in table 1 for these three subjects. A comparison of these three cases with those read directly from the Du Bois height-weight chart indicates that the measured body surface is slightly greater than that estimated from the chart. Thus, in the case of subject 6 the area estimated from the chart is 0.04 sq. m. lower than that actually measured; in the case of subject 7 it is 0.06 sq. m. lower; and in the case of subject 8 it is 0.05 sq. m. lower. In other words, the surface areas as actually measured were uniformly about 3 per cent greater than the areas estimated from the chart.

Physiological functions: Vital capacity. With these 9 subjects the vital capacity ranged from 2.00 to 2.70 liters, being on the average 2.21 liters. There has been a disposition in recent years, especially in the clinic, to use the vital capacity as an index of physical fitness. Thus, based upon a large number of measurements of normal American men and women, it has been computed that there is a close correlation between the vital capacity and the total stature, on the one hand, and the vital capacity and the surface area, on the other hand. West (14) computes that for women (hospital nurses) the vital capacity in cubic centimeters divided by the height in centimeters equals 20.0, and that the vital capacity in liters divided by the body surface in square meters is equal to 2.0. The Teachers College group (36 women) showed ratios of 18.3 and 1.86, respectively. These relationships are also worth considering in the case of our Orientals, as evidence of their condition. In the case of our subjects the vital capacity divided by the height ranged from 13.1 with subject 4 to 17.0 with subject 7, the average of all 9 subjects being 14.3, instead of 20.0 as suggested by West. The vital capacity divided by the surface area ranged from 1.36 with subject 3 to a maximum of 1.74 with subject 7, the average for all 9 girls being 1.54 instead of 2.0 as suggested by West. Thus, based on these American standards, our subjects showed a very low vital capacity. Foster and Hsieh (5) suggest for Chinese women a normal standard of 1.5 for the relationship between body surface and vital capacity, and 14.0 for the relationship between vital capacity and height. Singularly enough these values, which are drawn from 75 women, agree exactly with our findings on our 9 subjects. One should accentuate the interesting point raised by Foster and Hsieh that there should be a special standard for Chinese men and women and that American standards may not be applied. We are indebted to Prof.

Abby H. Turner of Mount Holyoke College (now temporarily at the Harvard School of Public Health in Boston) for a statement of some of her findings with two Chinese women, three Japanese women and one Siamese woman, all students in Boston. Comparing them with over 1,300 American students, she finds that their vital capacity is very low, quite in the order of that noted by us and by Foster and Hsieh.

Blood pressure. With only three of our subjects, nos. 6, 7 and 8, was the blood pressure determined. The systolic pressure was 98, 103 and 102 mm., and the diastolic pressure was 62, 71 and 68 mm., with subjects 6, 7 and 8, respectively. The pulse pressure was 36 mm., 32 mm. and 34 mm., respectively. While the pulse pressures are slightly lower than what is accepted as normal, we believe they are not significant.

Pulse rate. In each of our experiments the pulse rate was frequently counted and those recorded in table 1 are the average pulse rates obtained during the periods of the minimum basal metabolism. The average pulse rate of all nine subjects was 60 beats per minute, the minimum being 54 and the maximum 64 beats. In general, when one considers that the pulse rates of women are higher than those of men, these pulse rates seem somewhat low. Thus, the average pulse rate of 90 women, whose metabolism data were analyzed by Harris and Benedict (12), was 68 beats per minute. Most of these women were of college age. A group of 121 men, also studied by Harris and Benedict, had an average pulse rate of 61 beats. The Teachers College (20 to 30 years) group had a pulse rate of 69 beats per minute. It would appear, therefore, as if these Oriental women had a pulse rate distinctly lower than the average. Professor Blunt and Miss Dye, however, have reported the pulse rates of 17 college women (whose average age, to be sure, was somewhat greater than the average age of our group), whose average minimum pulse rate was 62 beats per minute. It is worthy of comment that the lowest rate recorded by them is 54 beats in the case of their subject I, a Chinese woman. This was also the minimum rate observed in our series. The general picture in the case of 9 Oriental women, therefore, is that of a pulse rate slightly, although not pronouncedly, low.

Respiration rate. Although it has not been the common custom to report the respiration rate in basal metabolism experiments, the rates recorded in table 1 for our subjects are for the most part quite similar to those noted with normal individuals with whom we have worked. Thus, the average respiration rate of 14 noted with these 9 Orientals is insignificantly higher than the average of the Teachers College group, i.e., 13.4 respirations per minute. One striking exception to this is the very low respiration rate of the Japanese, no. 8, i.e., 9 respirations per minute. These rates are the averages of the respiration-rates throughout the entire experimental period of 8 to 10 minutes' duration. Individual

counts at different periods of the experiment in the case of subject 8 showed not infrequently a rate as low as 6 respirations per minute. When this subject was breathing normally in the room, without mouthpiece, the rate was 13 respirations per minute. It is important to point out here the possibility of a pronounced effect of the use of any respiration appliance upon the respiration rate. With some subjects, at least, the introduction of a respiration appliance, such as a mouthpiece, may so completely distort the normal respiratory rhythm as to cause abnormal ventilation of the lungs, with a pronounced effect upon the respiratory quotient. The respiration rate of 6 noted with subject 8 on at least two different counts is lower than any respiration rates of women that we have found recorded in the literature, and also lower than those recorded for men save for one exception, singularly enough noted in the Nutrition Laboratory. Benedict and Cathcart (15), in studying several subjects in connection with muscular work experiments, noted with one subject, W. G. A., in three different respiration experiments ranging from 10 to 14 minutes, a respiration rate in the post-absorptive condition of 4.8, 4.7 and 4.6 respirations per minute. A pronounced alteration in the respiration rate when using a mouthpiece, usually a slowing of the rate, has frequently been noted by us and is not infrequently noted in the literature. Thus, Professor Zuntz (16), in metabolism experiments employing a mouthpiece, frequently showed a respiration rate as low as 6.

Basal metabolism. The basal metabolism of these college women was computed from the measured oxygen consumption per minute, which is recorded in one of the columns of table 1. The oxygen consumption varies from 146 cc. with subject 9 to 187 cc. with subject 3. The total 24-hour heat production has been computed from these oxygen figures, assuming an average respiratory quotient of 0.82 and a calorific value of oxygen of 4.825 calories per liter (17). This ranges from 1,014 calories with subject 9 to 1,299 calories with subject 3. The average for all 9 subjects is 1,147 calories per 24 hours. A comparison of the total 24-hour heat production of the various members of this group and particularly a comparison of the metabolism of these Orientals with that of other individuals cannot be made intelligently, however, without reference to one or more of the commonly accepted standards for basal metabolism. The average total heat production of this group of 1,147 calories is, as a matter of fact, measurably less than the average of 1,349 calories found with the 103 women analyzed by Harris and Benedict (12), and the average of 1,315 calories found with the 43 women between 20 and 30 years in the Teachers College series. On the other hand, as has already been pointed out, these two groups of normal American women were both taller and heavier on the average than were these Orientals. The

fact that, of these latter, three subjects had a 24-hour heat production of less than 1,100 calories is striking, but in consideration of their small body-weights, there is no unusual picture definitely shown by this method of comparison.

In comparing these Orientals with normal American women, we wish to point out what we have observed rather frequently in recent years, not only in our own somewhat extended, unpublished results but in those of others, that is, that the so-called "standards" for women are obviously somewhat too high. Practically any group of young college women will show an average basal metabolism somewhat less than the standard, with, however, many individuals in such a group showing values above the standard. We will, nevertheless, refer these measurements to the large series of standard measurements analyzed by Harris and Benedict, from which their prediction formulas were derived. The formula for women (12), which has been published many times, is as follows:

$$h = +655.096 + 9.563w + 1.850s - 4.676a$$

In this formula h equals the heat production per 24 hours, w equals the weight in kilograms, s equals the stature in centimeters, and a equals the age in years. To facilitate the computation of the predicted metabolism a series of tables was prepared by Harris and Benedict, which have been subsequently reprinted in many places (18).

By means of the Harris and Benedict prediction formula for women we have predicted from the age, height and weight of our several subjects their total 24-hour metabolism and compared it with that actually measured. The deviations from these predictions are also recorded in table 1, expressed on the percentage basis. In this comparison the striking point noted with all of the subjects is that the metabolism as actually measured is invariably somewhat less than the predicted metabolism, the average deficit in the 9 cases being 10.4 per cent. Six cases are 9.9 per cent or more below normal.

Owing to the somewhat abnormal configuration of the bodies of these Orientals when compared with American women, those who are inclined to discredit the validity of the Harris and Benedict formulas may reasonably take the position that with these subjects the Harris and Benedict formula may not obtain, and that one should turn to the other commonly accepted standard, that of Aub and Du Bois (19). This standard for the age range of our subjects, namely, between 20 and 30 years, is 37.0 calories per square meter of body surface per hour. Accordingly, for comparison with this standard we have divided the total 24-hour metabolism of our subjects by the surface area and by 24 to obtain the heat production per square meter per hour. Thus, with subject 1 the heat

production is 35.8 calories per square meter per hour. With all of the subjects except nos. 6, 7 and 8 the surface area as taken from the height-weight chart was used. With these three subjects the actually measured surface areas were used. A comparison of the heat production per square meter of body surface per hour with the Aub and Du Bois standard shows that the percentage deviations from this standard are of almost exactly the same order of magnitude as were the percentage deviations from the Harris and Benedict standards. Thus, the average deviation from the Aub and Du Bois standard is −10.2 per cent, as compared with −10.4 per cent in the case of the Harris and Benedict standards.

Those who might be inclined to question the validity of the body surface as determined from the Du Bois height-weight chart when applied to Chinese and Japanese can only be referred to the discussion of the measurement of surface area given earlier in this paper (see page 455), where it was pointed out that the actually measured surface areas from the linear, anatomical measurements were only about 3 per cent higher than those obtained from the height-weight chart. The disproportion between the actually measured surface area and the surface area as indicated by the height-weight chart cannot possibly explain, therefore, the predominantly low metabolism exhibited by practically all of these subjects.

The metabolism as predicted by Dreyer's method (20) has also been computed, although the values are not recorded in table 1. The deviations from the metabolism predicted on this basis were also found to present the same picture, the metabolism of all of the subjects being below the predicted metabolism.

An examination of the physiological data for our subjects shows that they are not abnormal as regards blood pressure, pulse rate and respiration rate, when individuals of a small size are considered. The state of nutrition, as evidenced by the pelidisi, is essentially normal. The vital capacity is abnormal, judged by American standards, but conforms fully with other standards obtained on Chinese and Japanese women. The mouth temperatures were all well within normal limits.

With subject 7 rather an extensive series of surface temperature measurements was made with the thermo-electric method (21). Similar measurements were made on other college girls at the same time. A careful examination of the data gives no hint that the surface temperature of this woman was in any way different from that of her college associates. Thus there were no abnormal physiological conditions so far as the surface area and the loss of heat from the surface of the body is concerned.

A part of the energy deficit which appears with all 9 subjects may undoubtedly be ascribed to what we believe to be an established fact, i.e., that all the present standards for women are probably 5 per cent too high.

Of the 9 Orientals considered in the above discussion, however, 7 are *more* than 5 per cent below standard. Thus, if the standard were lowered (as we think it ultimately will be) by 5 per cent, our general thesis would hardly be affected. By such a lowering of the standard, however, two important points are accentuated; 1, the difference between the two sexes would become the significant one of 12 per cent, and 2, those clinicians bound by the metabolic fetish of accepting deviations of plus or minus 10 per cent from the standard as the limits of normal metabolism may now find that women formerly showing a metabolism of +9.9 per cent really had a metabolism of +14.9 per cent and hence mathematically, if not clinically, become pathological material.

In spite, however, of any proposed changes in the standard level, it is clear that with all of our Orientals the picture is persistently that of a metabolism lower than the present normal standards, and in at least 7 cases appreciably below the suggested change in level. We believe we are thoroughly justified, therefore, in emphasizing the high probability that the low metabolism exhibited by this group of Oriental women may be considered as a racial characteristic. This belief is substantiated by the earlier study of Professor Blunt (1) and the unpublished work of Earle (7), although his observations were made under Oriental conditions of life so far as diet and climatic environment are concerned.

After the completion of this research and after this manuscript had been prepared, we received a report from the Imperial Nutrition Institute of Japan, kindly sent by its director, Prof. Tadasu Saiki. This report was published in Tokio in October, 1924, but was printed wholly in Japanese. Thanks to the assistance of Doctor Tamiya at the Harvard Medical School, portions of this report were translated and we find that Dr. Takahira and his associates in Prof. Saiki's Institute have studied many Japanese in a clinical respiration chamber (22), among others 16 women teachers.[1] The average values for these 16 women are as follows (23):

Age	34 years, 1 month
Body weight (presumably nude)	46.88 kgm.
Height	149.9 cm.
Surface area	1.39 sq. m.
Total 24-hour heat production	1,043 cals.
Heat per kilogram per 24 hours	22.26 cals.
Heat per square meter per hour	31.46 cals.

Comparing the heat production per square meter of body surface per hour, as noted with these 16 women, with the Aub and Du Bois standard and with our own average value of 33.2 calories per square meter, we find

[1] After this article went to press we received from Dr. Takahira the full report of his extraordinary series of observations, in which the basal metabolism of 120 Japanese men and women was studied.

that the Tokio experiments are wholly in agreement with our observations on Orientals living under American conditions.

The explanation of these phenomena by the most popular present-day expedient, i.e., endocrine inequalities, is left to others. Suffice to say that no evidence of abnormality of development, pubic hair, etc., was apparent in any of our cases. The facts are first to be established, and we believe that the data show that Oriental women have a metabolism per square meter of body surface 10 per cent lower than the commonly accepted standards for American women and consequently 17 per cent lower than the standards for men.

SUMMARY

Metabolism measurements are reported on 9 normal Oriental women, 7 Chinese and 2 Japanese, of an age ranging between 21 and 29 years, transplanted to an American climate and food conditions, and surrounded by the nervous tension incidental to the rush of Western civilization. The findings may be summarized as follows:

1. The vital capacity of these Orientals was very low, i.e., on the average 14.3 cc. per centimeter of height and 1.54 liters per square meter of surface area.

2. The blood pressure determined on 3 of the subjects was normal.

3. The average pulse rate for the entire group was 60 beats per minute, with a minimum of 54 and a maximum of 64, values that are at the lower, if not below the lower, limits for normal American women.

4. The respiration rate varied from 9 to 18 respirations per minute, averaging 14. One subject had a very low rate of 6 respirations per minute for a few minutes while breathing through a respiration apparatus, although her rate when not breathing through the apparatus was 13.

5. The pelidisi ranges from 88 to 99, averaging 92; with a slight tendency to be somewhat lower than the presumed normal of about 95.

6. The basal metabolism was in most cases strikingly low, on the average being 10.4 per cent below the Harris and Benedict prediction standard. Comparisons with the Aub and Du Bois and with the Dreyer standards give essentially the same picture, i.e., a persistently low metabolism in these Orientals. Since there was nothing in the physiological measurements of these young women to indicate abnormality, we are forced to the conviction that we are dealing with a specifically low cell metabolism characteristic of the Orientals, and that American and English physiological standards may not be applied to Orientals, particularly in pathological cases. Judging from the scattered reports of observations of basal metabolism in the Orient, we believe that our data show that transplanted Orientals retain their inherited low metabolism.

7. An examination of data obtained in our several laboratories (but not here published) and data published elsewhere on normal women leads

us to the belief that the present standards for American women are too high, indeed, approximately 5 per cent too high. Consequently those who adhere to a standard of plus or minus 10 per cent as an index of normality may have to realize that the metabolism of all women found to be between +5 and +10 per cent of the present-day standards is really between 10 and 15 per cent above the true normal.

8. The standards for basal metabolism have already, by common consent, been modified for the effects of sex and age. It is now suggested that the correction for difference in sex be increased and that a racial effect in the direction of a lower metabolism be recognized with the Chinese and Japanese.

BIBLIOGRAPHY

(1) BLUNT AND DYE: Journ. Biol. Chem., 1921, xlvii, 69.
(2) YANO: Mitteilungen aus d. med. Fakultät d. kaiserl. Univ. Kyushu, Japan, 1920, v, 189.
(3) CADBURY: Arch. Intern. Med., 1922, xxx, 362; Ibid., China Med. Journ., 1922, xxxvi, 170; Ibid., China Med. Journ., 1923, xxxvii, 715, 823.
(4) CRUICKSHANK: China Med. Journ., 1923, xxxvii, Supp. 12, 1.
(5) FOSTER: China Med. Journ., 1924, xxxvii, 285; also, FOSTER AND HSIEH: Arch. Intern. Med., 1923, xxxii, 335, 342.
(6) FLEMING: Journ. Metab. Research, 1923, iv, 105.
(7) EARLE: The Caduceus, 1922, i, 85.
(8) EIJKMAN: Journ. de Physiol. et de Path. gén., 1921, xix, 33.
(9) KNIPPING: Arch. f. Schiffs- und Tropen Hygiene, 1923, xxvii, 169.
(10) ALMEIDA: Journ. de Physiol. et de Path. gén., 1924, xxii, 12.
(11) DU BOIS AND DU BOIS: Arch. Intern. Med., 1916, xvii, 863.
(12) HARRIS AND BENEDICT: Carnegie Inst. Wash. Pub. no. 279, 1919.
(13) BENEDICT: Proc. Amer. Philos. Soc., 1924, lxiii, 25 and 30.
(14) WEST: Arch. Intern. Med., 1920, xxv, 306.
(15) BENEDICT AND CATHCART: Carnegie Inst. Wash. Pub. no. 187, 1913, table 35, 47.
(16) ZUNTZ, LOEWY, MÜLLER AND CASPARI: Höhenklima u. Bergwanderungen, 1 Aufl., Berlin, 1906, table xv.
(17) CARPENTER: Carnegie Inst. Wash. Pub. no. 303A, 1924, table 14, 105.
(18) HARRIS AND BENEDICT: Carnegie Inst. Wash. Pub. no. 279, 1919, tables I to IV, 253–266; CARPENTER: Carnegie Inst. Wash. Pub. no. 303A, 1924, tables 24 to 27, 110–121; GRAFE: Die pathologische Physiologie des Gesamtstoff- und Kraftwechsels bei der Ernährung des Menschen, Munich, 1923, tables I to IV, 488–499; BENEDICT: Abderhalden's Handbuch der biologischen Arbeitsmethoden, 1924, Abt. iv, Teil 10, tables 21 to 24, 657–674; ROTH: Metabolimetric compendium, Battle Creek, Michigan, 1924, 2.
(19) AUB AND DU BOIS: Arch. Intern. Med., 1917, xix, 831.
(20) DREYER: Lancet, 1920, Part 2, 290; also, CARPENTER: Carnegie Inst. Wash. Pub. no. 303A, 1924, table 29, 122.
(21) BENEDICT, MILES AND JOHNSON: Proc. Nat. Acad. Sci., 1919, v, 218; also, BENEDICT: Asher-Spiro's Ergebnisse der Physiologie, Supplement Band, (Festschrift für Leon Asher), 1925.
(22) BENEDICT AND TOMPKINS: Boston Med. Surg. Journ., 1916, clxxiv, 857, 898, 939.
(23) TAKAHIRA, KITAGAWA, ISHIBASHI AND KAYANO: Report of the Imperial Nutrition Institute, Tokio, Japan, 1924, 88.

THE RELATION BETWEEN THE SECRETORY AND MOTOR ACTIVITY IN THE FASTING STOMACH (MAN)

FREDERICK HOELZEL

From the Hull Physiological Laboratory of the University of Chicago

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In the course of a study to determine whether gastric acidity is a factor in the complex of hunger sensation, an apparent relation between secretory and motor phenomena of the fasting stomach was observed.

Bearing upon this subject, Carlson (1912) found that the mucin was increased and the free HCl decreased in the gastric secretion during strong contractions in a gastric fistula subject. Carlson (1916) also reported that the continuous secretion in the same subject varied in quantity from a few cubic centimeters to 60 cc. per hour and that the acidity varied in proportion to the rate of secretion. Ivy found that the gastric secretion was greater in quantity (1925) and higher in acidity[1] during gastric motor activity than during quiescence. Jarno and Heks (1920), relying upon the symptom of borborigmi as evidence of gastric motility, stated that the contents of the fasting stomach were generally small in quantity after periods of motility and large after quiescent periods. Rehfuss and Hawk (1921) believed that the pylorus is relaxed during the interdigestive phase of gastric function because of *a*, frequent evidence of duodenal regurgitation, and *b*, fluoroscopic findings. Boldyreff (1911) observing fasting dogs with gastric fistulas, reported that alkaline mucus was discharged from the stomach with the periodic motility which he observed and that this periodic motor activity was suspended when a spontaneous secretion of gastric juice began after about 24 hours of fasting. Anitschkow (1924), also using gastric fistula dogs, found that while no gastric juice was secreted during quiescence and during the periodic mild motility which Boldyreff observed, gastric juice was secreted freely during the stronger (more continuous and higher tonus) motility periods.

In regard to these findings, it is of interest to note that Carlson's and Boldyreff's observations with reference to mucous secretion and decreased acidity with gastric contractions agree with one another but it seems difficult to reconcile these observations with Ivy's findings on himself. The report of Jarno and Heks leaves the wish for more objective evidence of gastric motility than borborigmi, as they may be of intestinal origin. The

[1] Personal communication.

conclusion of Rehfuss and Hawk that the pylorus is relaxed in the fasting stomach is not supported by a sufficiently uniform finding of regurgitation and the fluoroscopic findings can not be relied upon because of the questionable specific effect of the introduced opaque material. I have shown elsewhere (1924) that such material (barium or bismuth) can not be expected to yield normal results in motility studies because of its high specific gravity. The observations of Boldyreff and Anitschkow on dogs raise the question as to what extent they apply to man and whether they obtained reliable data on gastric secretion by the technique which they employed.

METHODS. Observations were made from 10 to 60 hours after eating. A modified Rehfuss tube was used in making the aspirations of the gastric contents. In order to maintain conditions in the stomach as nearly normal as possible, the gastric contents were promptly returned (via the tube) after noting the quantity of each aspiration, retaining only 1 or 2 cc. for titration. Aspirations were made as infrequently as possible consistent with obtaining the desired data. The tube was usually withdrawn after returning the gastric contents so as to minimize the possible abnormal effect of intubation during the intervals between aspirations. However, in aiming to obtain accurate data on the rate of secretion, practically continuous aspiration was resorted to. In all cases attempts were made to prevent the swallowing of saliva during intubation. As the subject (the writer) had previous experience in using introspection in connection with the balloon method of study of the hunger contractions (Carlson, 1918), the subjective sensations were at first relied upon as evidence of gastric motility. However, the subjective findings were later confirmed by using the balloon and aspiration tube alternately.

RESULTS: *Quantity and motility.* The contents of the fasting stomach were found to vary in quantity from practically nothing to 135 cc. Confirming the observation of Jarno and Heks, the gastric secretion was found to accumulate in the stomach during motor quiescence and to be discharged during gastric motility. The bulk of the accumulated gastric secretion usually left the stomach with moderate tonus rhythms, that is, before definite gastric contractions became evident. Usually less than 10 cc. of juice was left in the stomach at the close of the hunger contraction periods (fig. 1). As the larger quantities were always secured during periods of motor quiescence, it would seem that this relation between motility and quantity of gastric content must be taken into consideration in any clinical evaluation of the content of the fasting stomach, particularly with reference to "hypersecretion." Provided the pylorus is normal, the content of the fasting stomach will be small if an aspiration is made toward the end of a period of hunger contractions and large if made toward the end of a quiescent period.

Mucus content. The gastric contents aspirated toward the end of the

periods of hunger contractions and frequently throughout the following period of quiescence were more highly viscid (richer in mucin content) and usually lower in acidity than the contents aspirated at other times (38 observations). This seems to confirm both Boldyreff's and Carlson's observations of mucus secretion with strong contractions. The observation of lower acidity with the increase in mucin content is also in harmony with the findings of Pasinetti (1905) and Klawansky (1923) that the viscosity of the gastric secretion is generally higher with the lower acid values.

Regurgitation. Regurgitation of the duodenal contents was never noted to take place during a period of gastric motor quiescence, that is, it never took place during a period which would seem to be the most probable time that the pylorus might be relaxed. Absence of regurgitation at such a time may indicate the absence of intestinal secretory and motor activity during gastric motor quiescence, which Boldyreff (1911) reported to be the case in the dog. However, it was found in this study that an increasing sense of fulness was usually experienced when the accumulated gastric secretion rose above 50 cc. This would seem to indicate that a closure of the pylorus permitted the accumulation of the gastric secretion and prevented its leaving the stomach. The opinion of Rehfuss and Hawk that the pylorus is relaxed in the fasting stomach is therefore not supported. It seems more probable that the pylorus functions during the interdigestive periods very much as it functions during the digestive periods in regulating the rate of discharge of the gastric contents and the amount of regurgitation.

Acidity. During this study of 10 weeks in which over 500 aspirations were made, the acidity of the basal secretion never rose above 0.2 per cent free HCl. Free acid was sometimes absent for periods of as long as 30 minutes but there was never complete anacidity. On some days the acidity was relatively high; on other days it remained characteristically low. However, it underwent periodic fluctuations on both the higher and lower levels. Sometimes the acidity of the gastric contents was at its lowest level at the close of a hunger period; more frequently, the lowest level was not reached until some time afterward (apparently coinciding with the time of greatest mucin content). Then the acidity would increase until regurgitation apparently checked further increase or caused a transitory decrease. Finally, the acidity would decline again with the increasing evidence of mucin in the gastric contents toward the close of the hunger periods (fig. 2). As the acidity was, on the whole, higher during the greater part of the motility periods than during quiescence, Ivy's observation on this point is, to this extent, confirmed.

Rate of secretion (and acidity). Judging the rate of secretion by the rate of its accumulation in the quiescent stomach, it varied from day to day from about 50 to 125 cc. per hour. Using continuous aspiration during

three observation periods totaling eight hours, the rate of secretion was found to be 80 to 100 cc. per hour. (All these figures include some swallowed saliva.) When the quantity aspirated by continuous aspiration was noted at successive intervals of 10 minutes or less, considerable fluctuation in the rate of secretion seemed to be indicated. However, when similar observations were made for successive intervals of 15 minutes or more, the rate of secretion seemed to be fairly uniform. The apparent

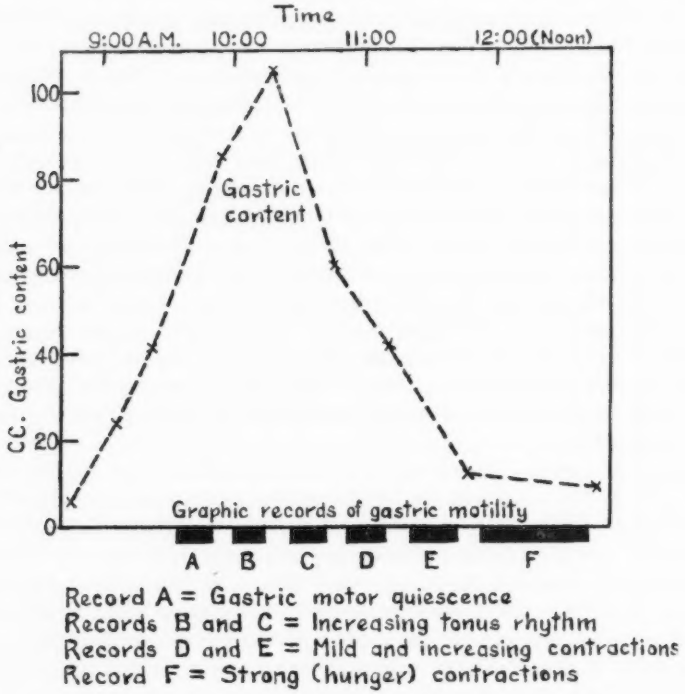

Fig. 1. (F. H.) Twelve hours after meal, showing accumulation of gastric basal secretion during motor quiescence and discharge of gastric content into intestine during period of increased gastric tonus and hunger contractions.

fluctuations in the rate of secretion for successive intervals of less than 15 minutes may be explainable as being due to the practical impossibility of making uniformly thorough aspirations during successive short intervals. In no instance could a definite relation between the rate of secretion and the degree of acidity be found. The acidity appeared to fluctuate independently of the rate of secretion (fig. 3).

Discussion. The data represented in figure 1 were obtained by the

alternate introduction and use of the aspiration tube and balloon. The balloon was withdrawn each time to facilitate the making of thorough aspirations; the tube was withdrawn after the contents were returned through it to minimize the possible effect of mechanical stimulation.

The hunger contraction periods indicated in figure 2 were noted subjectively. The data obtained did not yield a representative curve for the gastric contents during these hunger periods. The curve for the gastric content parallel with the hunger contractions in figure 1 is more representative of the usual findings. The curve for acidity in figure 2 shows typi-

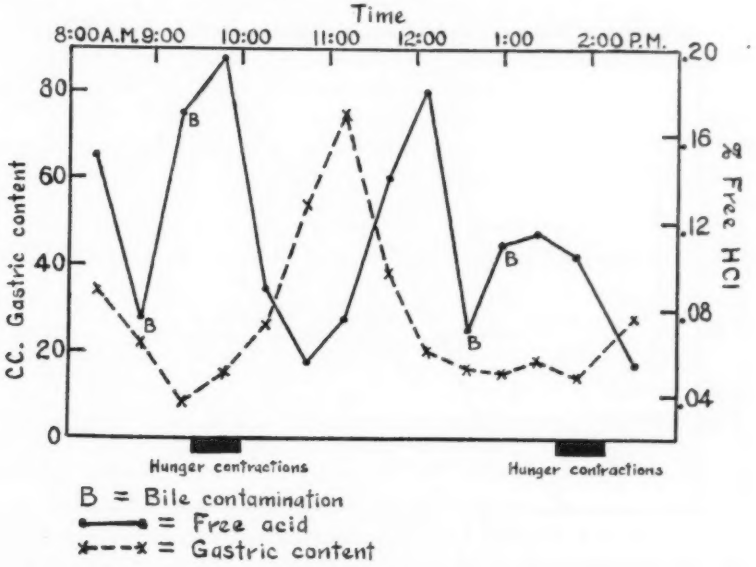

Fig. 2. (F. H.) Twelve hours after meal, showing decreased acidity with duodenal regurgitation and toward the end of, and after, periods of gastric hunger contractions.

cal fluctuations and is of a moderately high acid type (for the subject studied and the period of observation covered).

The data for figure 3 were obtained by continuous aspiration immediately following the period illustrated in figure 2. It will be noted that the acid curve in this figure is lower than what would be expected from the control period preceding it (fig. 2). Observations in the other studies made by continuous aspiration also indicated that the continuous intubation with the removal of the gastric contents (without restoration) modified the subsequent sequence of gastric motor and secretory function. How-

ever, the necessity of practically continuous aspiration in studying the rate of secretion was emphasized by the finding that as much as 100 cc. of the accumulated gastric secretion sometimes left the stomach within 20 minutes. Failure to find any definite relation between the rate of secretion and the degree of acidity of the basal gastric secretion does not exclude the possibility that such a relationship exists. But the findings of this study question the existence of such a relationship in all instances and emphasize the difficulty of proving that such a relationship exists in any case (in man).

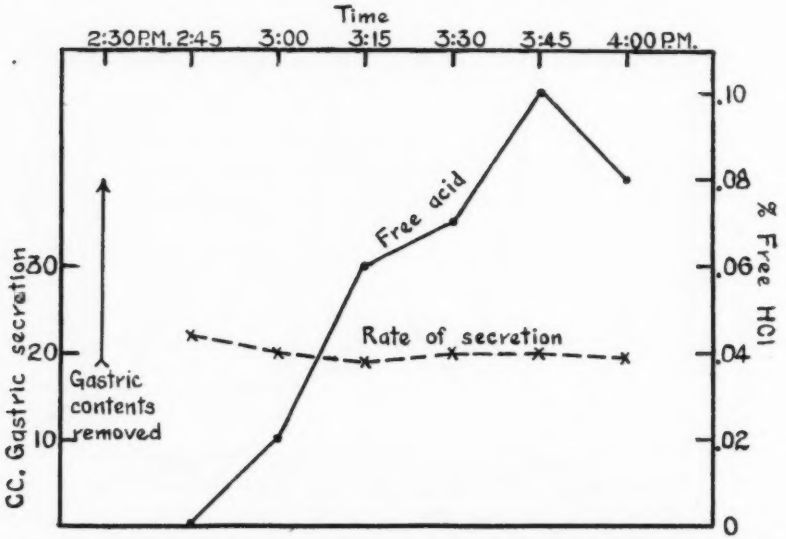

Fig. 3. (F. H.) Eighteen hours after meal. Period of apparent gastric motor rest (no regurgitation). Continuous aspiration of gastric content. Showing change in acidity with a practically constant secretion rate.

The rather uniform finding of a relative increase in mucin in the gastric contents after the stronger contractions seems to point to a causal relationship. It will be noted (fig. 1) that the stronger contractions are present when the contents of the stomach are apparently not large enough to serve as an effective (yielding) buffer between the walls of the contracting stomach. Hence, it seems possible that an increased secretion of mucus may be caused by irritation of the mucosa in consequence of the rubbing incident to the stronger contractions, that is, an increased secretion of mucus may be initiated by a type of mechanical stimulation which is regarded as a normal stimulus to such a secretion. If this is the case, it also follows that a mechanism is pointed to whereby an increase in the

secretion of mucus postulates a decrease in the secretion of acid juice. Else how are we to explain the finding of a fairly constant total gastric (mucoid plus acid juice) secretion? Such a mechanism would also explain the findings in gastric achylia, gastritis and experimentally induced mucus secretion as well as Boldyreff's and Carlson's findings of the absence or decrease of acid with evidence of increase in mucus.

CONCLUSIONS

1. The contents of the fasting stomach are likely to be small if aspirated toward the end of a period of hunger contractions and large if aspirated toward the end of a period of motor quiescence.

2. Periodic increases in the percentage of mucin in the fasting gastric contents appear after strong hunger contractions.

3. Periodic fluctuations in the acidity of the fasting gastric contents take place, and may be due to *a*, an increase or decrease in the mucin content; *b*, decrease or increase in the acid secretion, or *c*, duodenal regurgitation.

Thanks are due to Doctors Carlson and Ivy for aid and suggestions.

BIBLIOGRAPHY

ANITSCHKOW. 1924. Zeitschr. f. d. gesammt. exper. Med., xlii, 405.
BOLDYREFF. 1911. Ergeb. d. Physiol., xi, 186.
CARLSON. 1911. This Journal, xxxi, 151.
CARLSON. 1916. The control of hunger in health and disease. Chicago.
CARLSON. 1918. This Journal, xlv, 120.
HOELZEL. 1924. The rate of progress of food residues through the bowel. Chicago.
JARNO AND HEKS. 1920. Wien klin. Wochenschr., xxxiii, 578.
KLAWANSKY. 1923. Arch. f. Verdauungskr., xxxi, 23.
LIM, IVY AND MCCARTHY. 1925. Quart. Journ. Exper. Physiol., xv, 13.
PASINETTI. 1905. Zeitschr. f. exper. Path. u. Therap., ii, 252.
REHFUSS AND HAWK. 1921. Journ. Amer. Med. Assoc., lxxvi, 564.

OBSERVATIONS UPON THE ACTION OF INSULIN ON THE BLOOD, WITH SPECIAL REFERENCE TO THE CAUSE OF THE CONDITION KNOWN AS HYPOGLYCEMIA

LEE FOSHAY

From the Department of Medicine, Lakeside Hospital and Western Reserve University, Cleveland, O.

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Since November, 1922, insulin has been constantly used in this hospital as an agent in the treatment of cases of severe diabetes mellitus. One of the most remarkable phenomena that attend its usage has been the so-called hypoglycemic reaction. We have observed a considerable number of these reactions. The majority of them occurred during the early experimental period, when the potency of the product was variable, and when because of inexperience we were unable to estimate the approximate dosage necessary to meet the individual requirements. When such reactions occurred, a sample of blood was taken by the house officer and sent to the laboratory for a blood sugar determination.

It is of these blood sugar determinations, from blood taken frequently at the height of these hypoglycemic reactions, that I wish to speak. A number of them indicated a dangerously low blood sugar, some as low as 0.03 per cent. However, the majority of blood samples when analyzed for sugar gave amounts that were still well above the upper normal limit for blood sugar, that is, above 0.15 per cent. Citation of one such instance will serve as an example of the remainder. A young adult male was admitted to the house with a blood sugar of 0.37 per cent. Thirty units of insulin were given as the initial dose. Fifty minutes later the patient complained of tremulousness, apprehension of danger, sensations of warmth, hunger and thirst, with extreme lassitude and muscular weakness. When seen he was very drowsy, perspiring copiously, with flushed, warm skin, bradycardia and muscular twitching of his eyelids. A blood sugar was taken immediately and the patient was given sugar in water and orange juice. The blood sugar was 0.18 per cent. It seemed difficult to interpret this as a hypoglycemic reaction. Experience in treating these reactions added another difficulty toward the acceptance of hypoglycemia as the cause, since we found that a small amount of orange juice alone or a piece of bread and butter, or in fact almost any kind of food that was at hand, caused the disappearance of all of the symptoms and signs in a surprisingly short time.

The action of insulin on the blood of the dog. During the course of an experiment designed to show the relation of the relative blood volumes to a steadily increasing hypoglycemia in normal dogs (1), twenty units of insulin were given intramuscularly as a convenient means of reducing the high, experimentally produced hyperglycemia. Thirty-four minutes after the injection the blood sugar was 0.54 per cent, the serum sugar 0.50 per cent, and the corpuscular sugar 0.613 per cent. Ten minutes later the blood sugar was 0.34 per cent, the serum sugar 0.54 per cent, and the corpuscular sugar 0.058 per cent! So far as I could judge, the dog presented no symptoms whatsoever. These results are shown graphically in figure 1.

For the first twenty-five minutes following the injection of insulin, glucose was allowed to run into the femoral vein at the rate of 5.2 grams per kilo body weight per hour, or a total of 50 grams. This was done for two reasons. In the first place, it was desirable to differentiate as far as possible between an insulin response and the customary changes that follow cessation of glucose administration. In addition it was considered that such a situation would be more nearly comparable to the conditions that exist in a severely diabetic patient at the time of the first insulin injection.

This very remarkable rearrangement of the distribution of glucose between the corpuscles and the blood serum could not possibly have resulted from discontinuance of glucose. If we accept the prevalent opinion that the non-electrolytes, urea and glucose, are carried in the blood in simple solution and are distributed passively between corpuscles and plasma, as the experiments of Michaelis and Rona (2) (on glucose) indicate, then such a conclusion is impossible. Also I have observed both in patients and in dogs that as the whole-blood-sugar increases there is a lag in the diffusion of glucose into the cells, so that the major portion of glucose is in the plasma. Conversely, when the whole-blood-sugar is decreasing, the serum contains less sugar than the erythrocytes, illustrating the reverse of the same diffusion lag (1). This latter is in accord with the view expressed by Macleod (3). Consequently I believe it is correct to attribute the redistribution of glucose in this instance to insulin.

As I have stated, this dog did not present any of the symptoms of "hypoglycemia," or at least none were observed. It became necessary then to produce a hypoglycemia in a dog in order to ascertain whether or not the same redistribution phenomenon would occur; also to determine whether or not an insulin reaction would occur in the absence of hypoglycemia but with a similar impoverishment of the corpuscular glucose, and the relation of the latter to the onset of symptoms.

Such an experiment is illustrated in figure 2. The dog was given 25 grams of glucose intravenously before the insulin was given. This

accounts for the initial blood dilution. Thirty minutes after the injection of 50 units of insulin the dog was very restless; it cried out spontaneously, became salivated, vomited and began to have tonic contractions of the muscles of all four extremities. At the very beginning of the reaction the blood sugar was 0.30 per cent, the serum sugar 0.44 per cent, and the corpuscular sugar 0.128 per cent. A little later, with the reaction well under way, the blood sugar was 0.21 per cent, while the corpuscular sugar had fallen to 0.098 per cent.

Glucose was given intravenously in order to save the dog. The amount was the same as that given before the experiment, and the length of time between the glucose and the final sample of blood was the same as that between the glucose administration and the first sample of blood. The 25 grams given before insulin elevated the blood sugar to 0.50 per cent, the serum sugar to 0.52 per cent, and the corpuscular sugar to 0.47 per cent. The 25 grams given during the time of action of insulin only elevated the blood sugar to 0.37 per cent. The serum sugar rose to 0.50 per cent, approximately the same as before, whereas the corpuscular sugar did not increase at all but remained at 0.166 per cent. A similar experiment was conducted on a third dog with almost identical results. For the sake of brevity, only the protocol is given.

Two conclusions may be drawn from the experiments just detailed. One is that most of the glucose as such rapidly disappears from the erythrocytes as a result of some influence of insulin. The other is that insulin causes some change to occur that prevents the cells from absorb-

Fig. 1. Showing the partition of glucose between serum and erythrocytes during a steadily increasing hyperglycemia, and the change due to insulin. Forty-four minutes after giving 20 units of insulin the blood sugar is 0.34 per cent, the serum sugar 0.54 per cent, and the corpuscular sugar 0.058 per cent! The relative blood volumes show the usual changes due to increasing and decreasing the concentration of blood sugar. Continuous glucose was begun at *A* and stopped at *C*. Insulin, 20 units, at *B*.

Fig. 2. The action of insulin upon hyperglycemia in a dog. Note the greatly increased reduction of sugar in the corpuscles as compared to that in the whole blood and serum. This dog had a severe insulin reaction. Observe that the low corpuscular glucose persists during the initial period of recovery. The relative blood volumes show the customary response to both increasing and decreasing the blood sugar. Glucose, 25 grams, at *A* and at *D*. Insulin, 50 units, at *B*. Insulin reaction began at *C*.

Fig. 3. An insulin reaction without hypoglycemia. The reaction began at the time represented by the vertical line for 50 minutes, and was at its height fifteen minutes later. At this time the blood sugar was 0.20 per cent, whereas the corpuscular sugar was zero! Glucose 25 grams, at *A*. Insulin, 25 units, at *B*. Insulin reaction began at *C*.

Fig. 4. Figures taken from a diabetic patient after 20 units of insulin, showing the disproportionate reduction of corpuscular sugar identical with that previously found in the dog. Insulin, 20 units, at *A*.

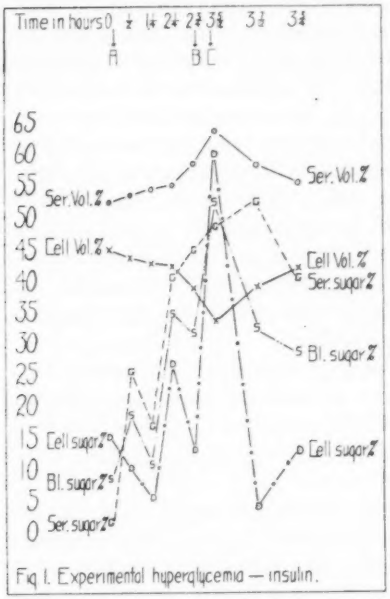
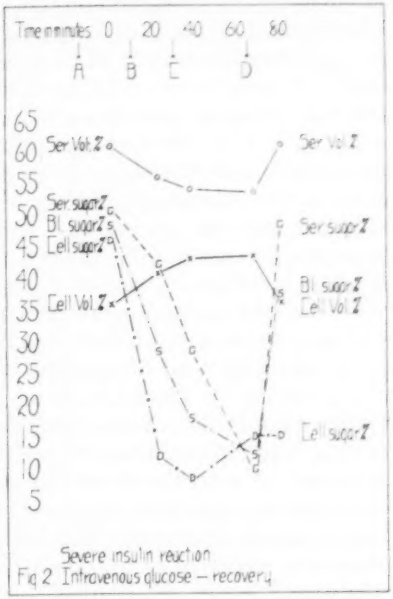


Fig 1. Experimental hyperglycemia — insulin.



Severe insulin reaction

Fig 2 Intravenous glucose — recovery

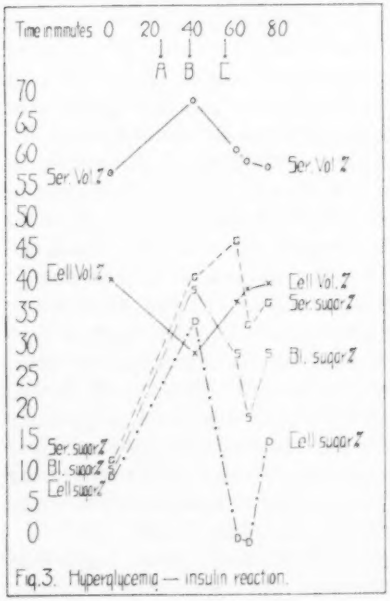


Fig. 3. Hyperglycemia — insulin reaction.

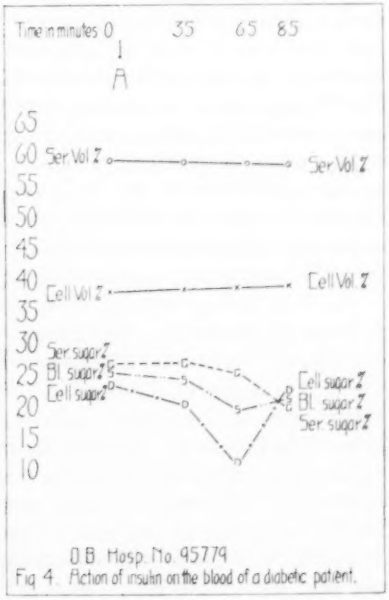


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Fig 4. Action of insulin on the blood of a diabetic patient.

ing glucose, even when it is added in great excess to the circulating plasma. It is not known whether this change takes place within the corpuscles or upon their surfaces or whether it represents a physical or a chemical change in the glucose. The fact is that it is rapidly reduced in quantity and may disappear entirely, as it seems to have done in the third dog.

So far as I am able to learn, glycogenesis has never been recorded as an event in the physiology of erythrocytes. Blood smears, made from each sample from the third dog, were stained with Best's carmine stain and examined for glycogen. None was demonstrable in the erythrocytes and it is not possible to say that there was any increase in carmine-staining substance in the leukocytes. In addition, there was not in the volume of the average erythrocyte the increase which one might expect to find if much glycogen had been deposited.

On the whole it appears unlikely that glycogenesis in the erythrocytes is responsible for the depletion of the corpuscular reducing-substance. On the other hand it seems highly probable that one of the physiological actions of insulin on blood is a deprivation or a reduction of the capacity possessed by erythrocytes to contain glucose. The experiments of Cori (4) suggested that this is true also of muscle cells. Macleod (5) has demonstrated quantitatively that after insulin has been given to rabbits there is a reduction in the concentration of glucose in the leg muscles. Although it is very entertaining to speculate upon what the result would be if this action of insulin occurred in brain cells or other nervous tissue cells, for want of such evidence one is not entitled to offer any general theory of the mechanism of the hypoglycemic reaction. It can be said that it appears to be associated with cellular structures and that in this respect it is similar to certain of the known oxidation-reduction systems (6).

The action of insulin on human erythrocytes. With this response to insulin established in dogs, it was desirable next to ascertain if it actually occurred in diabetic patients. To this end single doses of from 20 to 30 units of insulin were given to carefully selected patients, after which the partition of glucose was determined in serial samples of their bloods.

The first patient did not receive enough insulin (20 units) to give any appreciable response. The second patient, with a blood sugar of 0.30 per cent, serum sugar 0.27 per cent, and corpuscular sugar 0.34 per cent, was found to have, one hour and five minutes after 30 units of insulin, blood sugar 0.23 per cent, serum sugar 0.21 per cent, and corpuscular sugar 0.22 per cent. This represents a reduction of 0.07 per cent sugar in the whole blood, of 0.06 per cent in the serum, and 0.12 per cent in the corpuscles. Or, roughly, there was twice as great a reduction of sugar in the corpuscles as in either the serum or the whole blood!

The third patient, with blood sugar 0.27 per cent, serum sugar 0.28 per cent, and corpuscular sugar 0.25 per cent, received 20 units of insulin.

One hour later the blood sugar was 0.21 per cent, serum sugar 0.26 per cent, and corpuscular sugar 0.126 per cent! In this instance the reduction of corpuscular sugar was twice that of the whole blood, and six times that of the serum. This is shown graphically in figure 4. These were the same responses as were observed previously in dogs, though to a lesser degree. The much smaller changes are probably due to the fact that in proportion to their body weights the patients received much less insulin. In no instance did any symptoms appear.

The relative volumes of serum and cells were calculated from the electrical conductivities of the whole blood and of the serum of each sample by means of the relation developed by Stewart (7). The sugar determinations on the whole blood and on serum were made by the method of Folin and Wu (8). The corpuscular glucose concentrations were not determined directly but were calculated from the above data by means of the equation:

$$\text{per cent cell sugar} = \frac{\text{per cent blood sugar} - (\text{per cent serum volume} \times \text{per cent serum sugar})}{\text{per cent cell volume}}$$

The writer wishes to make grateful acknowledgment to Dr. G. N. Stewart and to Dr. C. D. Christie for their kindly advice and valuable criticism throughout the course of the work, and to Miss R. Trump for all of the chemical determinations.

SUMMARY

It has been shown that insulin causes a reduction in the quantity of glucose in the dog's erythrocytes disproportionate to the reduction in the whole blood, and that it causes the same response in human blood.

Moreover, it has been shown that insulin reactions can occur without hypoglycemia both in man and in the dog, and that such reactions in the dog begin simultaneously with the reduction of the corpuscular glucose. This offers a probable explanation of our failures to find the blood sugar low in the majority of our cases of insulin reactions, for we measured only the whole-blood-sugar.

The train of symptoms and signs that follows administration of large doses of insulin, and at least the early subjective and objective phenomena of insulin overdosage, are not associated with a hypoglycemia *per se*, but rather with a cytoglycopenia—a status of glucose impoverishment within the cell. This is of importance in the treatment of diabetes, especially when large doses of insulin are used. For, as I have shown, the whole-blood-sugar determination, which is commonly used as the therapeutic guide, is not a reliable index of an approaching insulin overdosage. This is particularly true of a patient in coma, when the symptoms are absent and the signs are obscured, for then another large dose of insulin, given because the last whole-blood-sugar was still quite high, may lead to a fatal issue.

Corpuscular sugar determinations are a reliable index of the physiological activity of insulin. They give evidence of degrees of action which are not obtained from whole-blood-sugar measurements.

PROTOCOL 1

TIME	GLUCOSE	K × 10⁴ AT 5°C.		SERUM	CELLS	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum					
				volume per cent	*volume per cent*	*per cent*	*per cent*	*per cent*
11:35		28.5	77.4	53.53	46.47	0.10	0.03	0.18
11:45	Begun							
12:06	(15.5 grams)	29.5	77.7	54.82	45.18	0.20	0.26	0.127
12:50	(37.0 grams)	30.8	78.9	55.98	44.02	0.13	0.18	0.066
1:50	(59.0 grams)	32.3	81.6	56.13	43.87	0.36	0.42	0.282
2:20	(84.0 grams)	32.0	77.4	58.78	41.22	0.33	0.46	0.145
2:26	Insulin 20 units							
2:45	(109 grams)							
2:51	Stopped. (140.0 grams)							
3:00		36.2	76.6	65.11	34.89	0.54	0.50	0.613
3:10		31.9	77.5	58.55	41.45	0.34	0.54	0.058
3:20		32.6	81.3	56.70	43.30	0.30	0.42	0.143

Dog 3. Female. Weight 23 kgm. Morphine narcosis. Continuous injection of 50 per cent neutral glucose solution by means of a Woodyatt apparatus. Total amount injected was 140 grams. Injection time 3 hours and 45 minutes. Glucose injected at a rate of 2.3 grams per kilo body weight per hour.

PROTOCOL 2

TIME	GLUCOSE	K × 10⁴ at 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR	PLASMA CO_2
		Blood	Serum								
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*	*volumes per cent*
2:00		34.8	80.0	60.56	39.44	6.685	59.0	0.13	0.10	0.17	62.0
2:15	Begun										
2:30	(50.0 grams)	44.9	74.2	78.20	21.80	4.920	44.3	0.60	0.68	0.312	58.0
2:45	(80.0 grams)	44.3	69.9	82.0	18.0	5.250	34.3	0.60	0.42	1.414	48.0
2:52	Insulin 20 units										
3:20	Stopped (97 grams)										
3:25		45.6	70.6	83.0	17.0	4.750	35.8	0.75	0.68	1.095	39.0

Dog 4. Male. Weight 9.5 kgm. No anesthesia. Continuous injection of 50 per cent neutral solution of glucose. Total amount injected was 97 grams, over an injection time of 70 minutes, representing a rate of 10.4 grams per kilo body weight

per hour. Diuresis of 650 cc. at 2:55. Urine contained 6.2 per cent glucose. Total fluid added to the circulating blood was 194 cc. The dog died during the evening of the same day.

PROTOCOL 3

TIME	GLUCOSE	K × 10⁴ AT 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum							
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*
11:45	25.0 grams									
12:00		31.9	72.7	62.38	37.62	7.085	53.1	0.50	0.517	0.470
12:10	Insulin 50 units									
12:22		31.4	78.4	57.0	43.0	7.780	55.2	0.30	0.428	0.130
12:37		31.1	80.2	55.5	44.5	8.090	55.0	0.21	0.300	0.098
1:05	25.0 grams									
1:08		30.5	79.4	54.96	45.04	8.020	56.1	0.136	0.111	0.166
1:20		31.8	72.3	62.57	37.43	6.840	54.7	0.375	0.500	0.166

Dog 5. Female. Weight 12 kgm. No anesthesia. Given 50 cc. of a neutral 50 per cent solution of glucose intravenously before the first blood sample was taken. Then given 50 units of insulin intramuscularly. A severe insulin reaction began at 12:30, reached the stage of clonic muscular spasms at 1:00, and was relieved by the intravenous injection of 25 grams glucose and 1 cc. of adrenalin subcutaneously.

PROTOCOL 4

TIME	GLUCOSE	K × 10⁴ AT 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum							
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*
12:15		32.9	79.4	58.6	41.4	7.450	55.5	0.12	0.12	0.12
12:40	25.0 grams									
12:52	Insulin 25 units									
12:54		38.6	74.6	70.0	30.0	5.610	53.5	0.40	0.42	0.353
1:13		34.6	77.5	62.2	37.8	6.340	59.6	0.30	0.48	0.003
1:19		34.1	79.4	60.2	39.8	7.070	56.3	0.20	0.34	0.000
1:28		32.9	78.7	58.8	41.2	8.070	51.1	0.30	0.380	0.161

Dog 6. Female. Weight 12.5 kgm. No anesthesia. Given 50 cc. of a 50 per cent solution of glucose neutral to phenolphthalein. Then given 25 units of insulin. A very mild insulin reaction began at 1:10, consisting of salivation, restlessness, muscular twitchings and irregular respirations. There were no muscular spasms or convulsions. The dog made a prompt recovery without glucose or other aid.

PROTOCOL 5

TIME	INSULIN	K × 10⁴ AT 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum							
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*
11:00		28.75	77.07	54.18	45.82	6.160	74.4	0.21	0.22	0.198
11:05	30 units									
11:30		28.91	76.68	54.70	45.30	5.560	81.5	0.23	0.25	0.205
12:20		27.94	75.35	54.19	45.81	5.740	79.8	0.21	0.17	0.257

Patient 1. Given 30 units of insulin. Lunch was eaten at 11:30, consisting of approximately two-fifths of a diet of P 75, C 75 and F 225.

PROTOCOL 6

TIME	INSULIN	K × 10⁴ AT 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum							
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*
10:45		29.36	73.62	57.68	42.32	5.210	81.2	0.25	0.27	0.223
10:55	30 units									
11:20		32.59	75.03	61.41	38.59	5.710	67.6	0.25	0.28	0.202
11:40		29.63	72.75	58.76	41.24	5.220	79.0	0.30	0.27	0.343
12:00		29.03	74.66	56.36	43.64	5.950	73.3	0.23	0.21	0.226

Patient 2. Given 30 units of insulin, after which three serial samples of blood were taken. Lunch, consisting of approximately two-fifths of a diet of P 75, C 75 and F 225, was eaten at 11:25.

PROTOCOL 7

TIME	INSULIN	K × 10⁴ AT 5°C.		SERUM	CELLS	RED BLOOD CELLS PER CUBIC MILLIMETER	AVERAGE RED BLOOD CELL VOLUME	BLOOD SUGAR	SERUM SUGAR	CORPUSCULAR SUGAR
		Blood	Serum							
				volume per cent	*volume per cent*	*millions*	μ^3	*per cent*	*per cent*	*per cent*
11:40		30.0	71.4	60.48	39.52	4.955	79.8	0.272	0.283	0.256
11:45	20 units									
12:15		29.5	70.9	60.1	39.9	4.630	86.2	0.258	0.283	0.221
12:45		28.6	70.0	59.6	40.4	4.700	85.9	0.208	0.263	0.126
1:05		29.2	71.4	59.4	40.6	4.485	90.5	0.234	0.230	0.239

Patient 3. Given 20 units of insulin. Lunch was eaten at 12:30. This consisted of about two-fifths of a diet composed of C 90, P 75 and F 220. Note the disproportionate reduction of glucose in the corpuscles in the second sample taken after the insulin was given.

BIBLIOGRAPHY

(1) Foshay, L. Journ. Exper. Med. (In press.)
(2) Michaelis, L. and P. Rona. Biochem. Zeitschr. 1908, xiv, 476: also Ibid., 1909, xviii, 375.
(3) Macleod, J. J. R. Physiol. Rev., 1921, i, 208.
(4) Cori, C. F. Journ. Pharm. Exper. Therap., 1923, xxii, 355.
(5) Macleod, J. J. R. This Journal. (In press.)
(6) Meyerhof, O. Chemical dynamics of life phenomena. Philadelphia, 1924.
(7) Stewart, G. N. Journ. Physiol., 1899, xxiv, 356.
(8) Folin, O. and H. Wu. Journ. Biol. Chem., 1919, xxxviii, 81.

RECIPROCAL INNERVATION AS A POSSIBLE LOCAL MECHANISM IN THE FROG

JOHN BETHUNE STEIN AND JOSEPH TULGAN

From the Laboratory of Physiology, New York University College of Dentistry

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In a recent publication (Stein and Tulgan, 1925) we made a statement to the effect that when the gastrocnemius muscle of the frog was directly stimulated, the tibialis anticus relaxed. In other words, reciprocal innervation of these two muscles was thus demonstrated. This phenomenon, which we were able to demonstrate so consistently, pointed to some local nervous mechanism, present at the knee joint, which seemed to be concerned with this reflex.

The results of this experiment seemed so novel that we decided to investigate this reaction under the various conditions enumerated below:

1. Brain pithed, cord and sciatic nerve intact.
2. Brain and cord pithed, sciatic nerve intact.
3. Brain and cord pithed, sciatic nerve cut in upper third of the thigh.
4. Brain and cord pithed and the sciatic nerve divided at its origin in the cord.
5. Brain and cord pithed and the peroneal branch of the sciatic nerve cut.
6. Entire leg amputated above the knee joint, with all muscles of the thigh removed.
7. Entire leg amputated above the knee joint, and novocaine injected into the joint and into the connective tissue surrounding the knee joint.

The object of doing the experiments in this order was to endeavor to eliminate all nervous connections above the knee joint which we thought might apparently play a part in this action. The observance of all these conditions was necessary in order to localize this phenomenon.

Experimental procedure: The gastrocnemius and the tibialis anticus muscles were carefully dissected out and "S" shaped hooks were inserted into their distal tendons. By means of pieces of thread these hooks were connected to the pullies of two muscle levers; the upper one for the gastrocnemius muscle, and the lower one for the tibialis anticus muscle. The levers were carefully balanced so that they were horizontal and wrote in the same vertical ordinate. The gastrocnemius muscle was after loaded but the tibialis muscle was loaded, but only by the weight of the lever, since the after loading screw of the lever to which the tibialis anticus was

attached was raised to permit the recording of the contraction or the relaxation of this muscle.

Sufficient care was taken to prevent any contact occurring between the muscles, usually 5 mm. separated the widest parts of the muscles. This precaution was taken to prevent any current from stimulating the tibialis anticus muscle when the gastrocnemius muscle was stimulated directly, and also to prevent any mechanical pull on the tibialis anticus muscle by the contracting gastrocnemius muscle. Although we separated the muscles to make sure that there would be no possibility of a leakage of electrical current to the tibalis muscle, we were certain that a relaxation of the tibialis could not occur even if some current did leak over, because direct stimulation of this muscle always gave contractions and never relaxations.

The animal was so tied down that any possibility of any mechanical factor playing any part was eliminated. All records were taken on a rapidly revolving drum.

Experimental results: 1. Brain pithed, cord and sciatic nerve intact: In this experiment the entire nervous system, with the exception of the brain, was intact. Direct stimulation of the gastrocnemius caused its contraction and also the relaxation of the tibialis muscle. Since the entire nervous system, with the exception of the brain, was intact in this case, one may draw many conclusions as to the centralization of this reciprocal action. From this experiment, the conclusion might be drawn that it is a pure reflex inhibitory action, and so it became necessary to eliminate other portions of the central and peripheral nervous system.

2. Brain and cord pithed, sciatic nerve intact: In this case the entire central nervous system was eliminated, leaving only the sciatic nerve or possibly some very delicate nerves, branches of the sciatic, which might reach the knee joint independently of the sciatic itself. On stimulating the gastrocnemius we observed again the same phenomenon as above. This led us to the conclusion that the central nervous system was not wholly responsible for the reciprocal innervation of these two particular muscles, and that the peripheral nerves might be responsible to a certain degree for this phenomenon.

3. Brain and cord pithed, sciatic nerve cut in upper third of the thigh: In this case the entire central nervous system was again eliminated, and the lower two-thirds of the peripheral portion of the sciatic nerve was left intact. The results obtained were the same as in all the cases enumerated above.

4. Brain and cord pithed and the sciatic nerve divided at its origin in the cord: The sciatic nerve was sectioned at the cord in this experiment in order to eliminate any fine branch that might travel independently of the sciatic and which might not be included in a section of this nerve lower down, as was done in the experiment above, and which might run inde-

pendently to the muscles of the knee joint. However, the phenomenon of reciprocal innervation persisted.

5. Brain and cord pithed and the peroneal branch of the sciatic nerve cut: In this experiment we endeavored to determine whether or not the peroneal branch of the sciatic played any part in this act, but direct stimulation of the gastrocnemius muscle still gave a relaxation of the tibialis, showing that this reaction was not dependent on either the sciatic or the peroneal nerves.

6. Entire leg amputated above the knee joint with all muscles of the thigh removed: This experiment was necessary in order to see whether this phenomenon could still be obtained when all possible anatomical connections, both muscular and nervous, were destroyed. If this phenomenon could still occur under these conditions, the only possible inference which can be arrived at, is that this particular phenomenon must be a local one confined to the knee joint; and, since we still obtained the same results under this condition, the inference that there is a local mechanism governing the reciprocal action of these two muscles appears to be proven, since all possible nervous and muscular connections were destroyed. Any reaction that would occur here must necessarily be of a local character.

7. Entire leg amputated above the knee joint and novocaine injected into the joint and into the connective tissue surrounding the knee joint: After injection of novocaine, the relaxation of the tibialis anticus ceased, although the gastrocnemius contracted as before. Since, from some of our previous experiments, we know that novocaine abolishes the irritability and conductivity of motor nerves, and since it is generally known that novocaine affects the sensory nerves and that it does not act directly on muscle, this local mechanism which is probably present in the knee joint or tendinous insertions of the muscles, must be of a nervous character; and that the contraction of the gastrocnemius when directly stimulated brings this local mechanism into play, causing a relaxation of the tibialis anticus. It is also possible that if the electrodes are placed close to the tendinous insertion of the gastrocnemius, the nerves of this local mechanism might be directly stimulated—thus also causing a contraction of the gastrocnemius and a relaxation of the tibialis anticus.

Discussion. In a series of experiments which we were doing, we noticed that every time the gastrocnemius muscle was directly stimulated by an electrical stimulus, the tibialis anticus muscle relaxed. Since this phenomenon was so striking, we decided to investigate it further and also to definitely locate the seat of this unusual action. It became necessary, therefore, to eliminate in some definite sequence portions of the nervous system that might be responsible for this occurrence.

We first eliminated the brain, then the cord, and finally the peripheral nerves to the muscles in question, and, finally, when all the nervous con-

nections to the joint were severed and we still obtained this result, the entire leg was amputated above the knee joint. However, the phenomenon still persisted, and this indicated that at the knee joint there was a mechanism which controlled locally this reaction. In order to determine whether this mechanism was of a nervous or muscular character, novocaine, which is known to abolish the irritability and conductivity of nerves but which has no affect on muscle directly, was injected into the knee joint and the surrounding connective tissue. After a lapse of a short space of time, direct stimulation of the gastrocnemius muscle gave a contraction but the relaxation of the tibialis anticus muscle was abolished. This clearly indicated the presence of some nervous mechanism within, or very closely surrounding, the knee joint which was responsible for this action.

Of course, we must not assume from these results that this mechanism is the only mechanism for the reciprocal action of these two muscles. It

Fig. 1. Reciprocal action of the gastrocnemius and tibialis anticus muscles when the leg is amputated above the knee joint.

Fig. 2. Shows the onset of the novocaine effect. The inhibition of the tibialis anticus is beginning to disappear.

Fig. 3. Shows the complete action of the novocaine where the inhibitory phase (relaxation of the tibialis anticus) has completely disappeared.

is probably subsidiary to a similar mechanism located in the central nervous system and that this lower local mechanism may be stimulated or inhibited by the higher one. It is possible that this is a local mechanism facilitating more rapid function, thus enabling the animal to perform quickly the acts of swimming, jumping, sitting, etc., and assisting greatly reflex actions. It probably diminishes the perceptibility of these acts. The natural movements at a joint are usually slow in proportion as the lever working at that joint is long. The natural movements at our shoulders and our knees are slower than at the digits. This local mechanism by facilitating more rapid function, may thus tend to counteract the natural slowness of action at a joint where the working lever is long. Such a mechanism assists greatly in the frog's usu status, usu situs and usu motus and possibly dimishes his perceptibility of posture and makes his reflexes more rapid.

The presence of these local mechanisms may facilitate a great many of our own actions, if such local mechanisms exist in higher animals.

Three tracings are submitted: figure 1 shows the reciprocal action when the leg is amputated. Figure 2 shows where the novocaine is acting and where the inhibitory phase is beginning to disappear. Figure 3 shows the complete action of the novocaine where the inhibitory phase (relaxation of the tibialis anticus has completely disappeared.

Further work on this question is in progress.

CONCLUSIONS

1. At the knee joint of the frog there is a local mechanism which is concerned with the reciprocal reaction of the gastrocnemius and the tibialis anticus muscles.

2. This mechanism is probably subsidiary to a higher mechanism in the central nervous system.

3. This local mechanism is of a nervous type, since the injection of novocaine in and around the knee joint abolishes it.

4. This reciprocal action of the two muscles involved could never be reversed, again indicating that the mechanism is of a nervous type.

BIBLIOGRAPHY

STEIN AND TULGAN. 1925. Science, lxi, 185.

ANALYSIS OF DAILY ACTIVITY OF THE ALBINO RAT[1]

JAMES ROLLIN SLONAKER

From the Department of Physiology at Stanford University, California

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In a previous paper (Slonaker, 1925) we showed that the female rat, during sexual life, exhibited marked rhythmic fluctuations in voluntary activity and that the spurts of activity occurred at the time of oestrus. We also showed that the rat performed almost all its activity during the night time. So far as we know, no finer analysis of the voluntary activity of the rat has been made. While we have demonstrated that there was a great increase of voluntary activity during oestrus as compared to that of dioestrus and a marked reduction during gestation and lactation, we did not know how the onset and duration of the activity was manifested.

This experiment was devised to show how the voluntary activity began and was distributed over each twenty-four hours during the different stages of the oestrual cycle and during pregnancy and pseudopregnancy. This was accomplished by taking hourly observations for a period of eight days and six hours. This enabled us to analyze the daily curve of activity into twenty-four component parts. Thirty-eight female rats, all in the prime of life, were used. These represented virgin and multiparous rats of various ages ranging from 148 to 375 days. Each animal was confined in a separate revolving cage. All the rats had been in their respective revolving cages for a considerable time and were accustomed to the environment and to turning the cages. Each cage was provided with an automatic registering device which recorded the number of revolutions turned. In order to insure that the readings of the number of revolutions turned were made at exact hourly intervals a clock, which made electric contact and rang a door bell hourly, was used. The cage readings were always taken and recorded in the same order immediately after the clock signal was given. From these data activity curves for each rat were plotted for the whole time. Many oestrual peaks were noted during the one hundred and ninety-eight hours of observation. In many of the rats oestrus occurred twice during this time. Forty typical oestrual peaks were recorded and used. Some of the females were pregnant and others proved

[1] This research has been conducted with the aid of the Department of Physiology and the Research Fund of Stanford University, and The Committee for Research on Sex Problems of the National Research Council.

later to be pseudopregnant. We were therefore able to analyze the daily activity of the rat at various ages during the different stages of the oestrual cycle, at various times during pregnancy, and during pseudopregnancy. None of the rats were nursing during this period of observation so we were unable to secure data relative to this condition. Several graphs representing typical activity of the above conditions will be given.

The feeding and watering of the rats was done between the hours of 5 and 6 o'clock p.m. At all other times the cages were not touched and the animals not disturbed.

The daily activity curves were plotted from the readings taken at the feeding time. Each circle in the curve of daily activity therefore represents the number of revolutions turned by the rat during the previous twenty-four hours. Since very few revolutions are turned during the daytime, the curve of daily activity practically represents the nightly run. A few curves of daily activity are given in order to designate and correlate the curves of hourly activity. In order to accustom the animals to artificial light during the night-time, the lights were left burning for several nights previous to taking the records and were also left burning each night of the experiment. In spite of this preliminary precaution the light stimulus had a more or less inhibitory effect in many cases on the voluntary running. The average amount of activity performed before and after the period of observations was somewhat greater than that during the period. The light stimulus not only reduced the amount of voluntary activity but also more or less disturbed the rhythmicity of the activity. As will be seen later, however, the artificial light stimulus did not noticeably alter the distribution of the activity during the twenty-four hours. The animals still remained nocturnal in their habits.

The food given was the same as had been used for a long time, and was always given in larger amounts than would be consumed before the next feeding time. It consisted of the following by weight: ground whole wheat, 3375; whole milk powder, 500; commercial casein, 750; sodium chloride, 50; calcium carbonate, 75; sifted ground alfalfa, 150; whole milk fat, 250. The dry ingredients were thoroughly mixed and the fat then stirred in. After reducing the lumps of fat to the smallest size by stirring, the mass was run through a grinding mill and then further stirred. In this manner we felt confident that all the ingredients were thoroughly mixed.

Time and duration of activity. There was a tendency on the part of most of the rats to show inquisitiveness when fed, but in the majority of the cases little or no running was done at that time. Some would turn the cage a few revolutions and then cease for one or more hours. Though an abundance of food was always present, the giving of new food caused them to eat freely for some time. After feeding many would curl up in their nests and sleep, others would be somewhat active, while a few would begin the nightly run.

There was a great variation in the time at which the nightly run was begun. It ranged from 3 p.m. to 2 a.m. In one case the activity was not begun until 7 am. and was continued for seven hours. This occurred during dioestrum. The majority of the animals began their activity between 8 and 11 o'clock p.m. This was true for all stages of the oestrual cycle. In figure 1 we have diagrammatically shown the average activity distribution of 40 cases of oestrum in continuous line and 48 cases of dioestrum in dotted line. Each of the circles represents the number of revolutions turned during the preceding hour. This figure shows that the

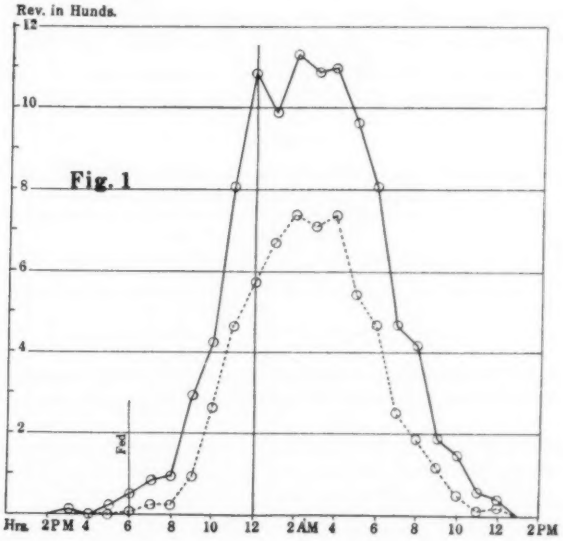


Fig. 1. Graph showing the average distribution of the hourly activity during the twenty-four hours of rats in oestrus, continuous line, and in dioestrus, dotted line. Each circle represents the average number of revolutions turned during the preceding hour.

main part of the activity occurred between the hours of 8 p.m. and 10 a.m. The parts of the curves before and after these hours represent a comparatively few cases in which the activity began early or late. It is also seen that the average length of the daily activity was one hour longer during oestrus than during dioestrus.

When the duration of the daily activity is considered we find that during oestrus it ranged from 8 hours to 21 hours and averaged 13.15 hours. The average run during oestrus was 10,429 revolutions. During dioestrus the range was from 5 hours to 19 hours and the average was 13 hours. In this

case the average run was 5136 revolutions. The average difference in duration of the activity was very slight. We thus see that the increased amount of activity during oestrus was due almost wholly to an augmented activity and very little to an extension of the activity period.

We have previously shown that the average length of the oestrual cycle for rats of a similar age to those in this experiment was four days. The peaks of activity, indicating oestrus, occurred with marked regularity each fourth day. The low points which occurred during dioestrus, however, were not necessarily so regular. In all cases the low points used represent dioestrus. For the purpose of convenience in analysis we have divided the oestrual cycle into four periods of one day each. The day preceding oestrus we have designated pre-oestrus; the day following oestrus, post-oestrus; the day following post-oestrus we have called dioestrus. This grouping is not intended to correspond to the different

TABLE 1

Showing the average number of revolutions turned for each hour of activity during the four successive days of the oestrual cycle

	OBSERVATIONS	SUCCESSIVE HOURS OF ACTIVITY																AVERAGE TOTAL
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Pre-oestrus...	31	196	557	603	906	1000	1052	894	718	576	302	275	271	130	66	18	40	7	7624
Oestrus....	40	269	620	780	1011	1029	1142	1180	1213	1041	693	553	408	150	67	61	99	32	10385
Post-oestrus...	30	228	587	806	742	937	855	670	503	380	264	197	99	55	21	10	2	1	6369
Dioestrus..	47	248	462	623	767	792	705	572	491	353	289	195	96	37	33	8	12	1	5690

stages of the oestrual cycle described by other authors. With the exception of the day on which oestrus occurred each of the other designated days may represent true dioestrual conditions. This arrangement, however, will enable us to show what occurred during each of the four activity periods of an oestrual cycle.

Owing to the wide variation in the time of beginning the daily activity, figure 1 fails to give a true average curve of a period of activity. In order to get a correct conception of the average activity of each of the four activity periods of an oestrual cycle we have rearranged the hourly readings of each rat under their appropriate headings so that the first, second, third, etc., hour's run was superimposed and averaged. Table 1 gives the average revolutions turned for each hour of the four activity periods during an oestrual cycle. It also gives the average total run for each of these periods. It is readily seen that during the pre-oestrual period the animals were more active than during post-oestrual activity, but that each

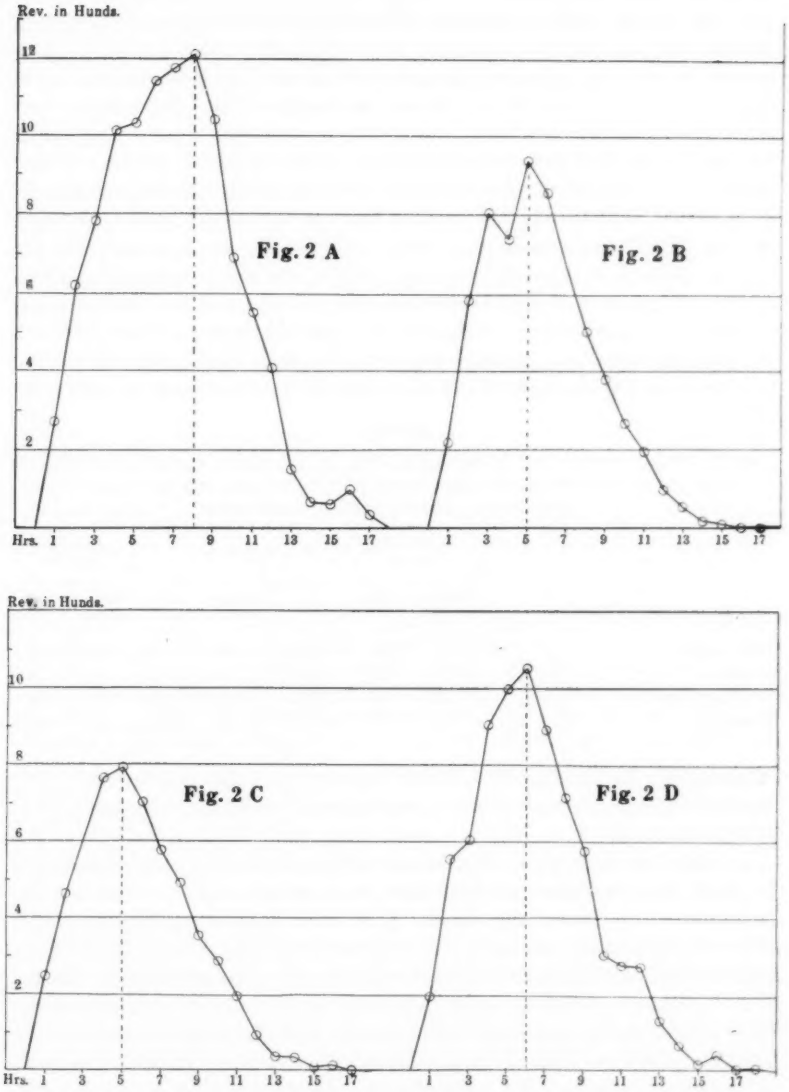


Fig. 2. Graph showing the average activity for successive hours during oestrus, A; post-oestrus, B; dioestrus, C; and pre-oestrus, D. Each circle represents the average number of revolutions turned during the preceding hour.

of these periods was greater than that of dioestrum. This seems to indicate that the activating hormone not only influences the activity during oestrus but also slightly augments that of the preceding and succeeding periods of activity, affecting the pre-oestrual more than the post-oestrual period. If this increased activity is due to the follicular hormone then there must be some inhibitory influences at work which prevent running during the daytime preceding and following the night of oestrus. These inhibitory influences are doubtless the same as those which make the rat a nocturnal animal. In our opinion light is one of the most important factors. By consulting figure 2, which is a graphic representation of the data in table 1, it is readily seen that the curve of pre-oestrual activity, *D*, resembles more closely that of oestrus, *A*, than either that of post-oestrus, *B*, or dioestrus, *C*. We also see that the curves of post-oestrual, *B*, and dioestrual, *C*, activity are very similar. They each reach the maximum at the same hour and show almost the same manner and time

TABLE 2

Showing the average per cent of total time, the average number of revolutions and the average per cent of total activity before and after the peak of activity of each of the four activity periods of the oestrual cycle

	PER CENT OF PERIOD		REVOLUTIONS			PER CENT OF ACTIVITY	
	Before peak	After peak	Total	Before peak	After peak	Before peak	After peak
Pre-oestrus	33	67	7,624	4,314	3,310	56	44
Oestrus	47	53	10,385	7,245	3,140	70	30
Post-oestrus	29	71	6,369	3,300	3,069	52	48
Dioestrus	29	71	5,690	2,892	2,798	51	49

of descent. In each of the periods the onset of the activity was very similar. It rapidly increased to a maximum after which there was a more gradual descent.

In table 2 we have given the per cent of the total activity period required to reach the maximum activity and the number and per cent of total revolutions run during this time. This table shows that during oestrus the peak of activity occurred only a short time before the middle of the activity period while in all the other cases it occurred much earlier. During the 47 per cent of the total time required for the rats in oestrus to reach their maximal run they turned 70 per cent of their total revolutions. In pre-oestrus 56 per cent of the total run was performed prior to the peak. In the other periods of the oestrual cycle, regardless of the variation in the number of total revolutions turned, they each had run approximately one-half of the total before reaching the peak of activity.

In order to make a comparison from a different angle we have computed

the average per cent of total activity performed at the end of successive hours for each of the four periods of the oestrual cycle. These data are given in table 3. By referring to this table it is readily seen that the rate of performing the work during the pre-oestrual and oestrual periods was very similar and differed markedly from that of the post-oestrual and dioestrual periods. We also see a close resemblance between the post-oestrual and dioestrual periods. In these two periods there was a greater tendency to crowd the bulk of the work into the early part of the period of activity than in the pre-oestrual and oestrual periods. Since we have often obtained successful matings in the pre-oestrual and none in the post-oestrual period we are led to conclude that the activating hormone was involved in the one case and only slightly or not at all in the other.

Activity of virgin rats. In figure 3 we have shown the daily activity and food consumption curves of a young virgin rat, E 4, at the age of 148 days. Each of the circles represents the number of revolutions and grams of

TABLE 3

Showing the average per cent of total activity performed at the end of successive hours for each of the four periods of the oestrual cycle

	OBSERVATIONS	SUCCESSIVE HOURS OF ACTIVITY																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pre-oestrus....	31	2.6	9.9	18.0	30	42	56	68	73	85	88	93.0	96.0	98.0	98.7	99.0	99.5	99.7
Oestrus.....	40	2.6	8.5	16.0	26	36	47	58	70	80	86	92.0	95.0	96.5	97.3	98.3	99.3	100.0
Post-oestrus...	30	3.6	12.7	25.0	37	52	65	76	84	90	94	97.0	98.0	99.3	99.5	99.8	99.9	100.0
Dioestrus.....	47	4.4	12.4	23.6	37	51	63	73	82	88	93	96.6	98.2	99.0	99.5	99.7	99.9	100.0

food eaten during the preceding twenty-four hours. This figure represents five oestrual peaks. In three of these our so-called post-oestrual day represents also the least amount of activity in that oestrual cycle. In the other two cases our so-called dioestrus was lowest in the cycle. In none of these does the pre-oestrual period occupy the lowest part of the curve. This curve also shows that the average activity for the pre-oestrual period was greater than that of the post-oestrual period. The distribution and amount of activity for each of the hours of the six days indicated by the numerals 1 to 6 are shown in figure 4 A, B and C. The open circles indicate that the animal failed to record a single revolution during the preceding hour. The solid circles represent activity, which in some cases consisted of only a few revolutions. Figure 4 A, December 14, and C, December 18, represent the hourly activity of this rat during the peaks 1 and 5 of figure 3. These two curves show how the activity was distributed during these two periods of oestrus. They show continuous

activity for ten and eleven hours respectively. They each show an initial spurt of two hours after which there was a decided reduction in activity. Following this slump there was a second more pronounced increase in the voluntary running which was continued for four or five hours before the general reduction began. This initial spurt and reduction in activity was found to occur in a large majority of the non-pregnant rats regardless of the day in the oestrual cycle. It is not, therefore, characteristic of oestrus. In some cases this initial spurt of activity was very great and was followed by only a slight retardation while in other cases it was low.

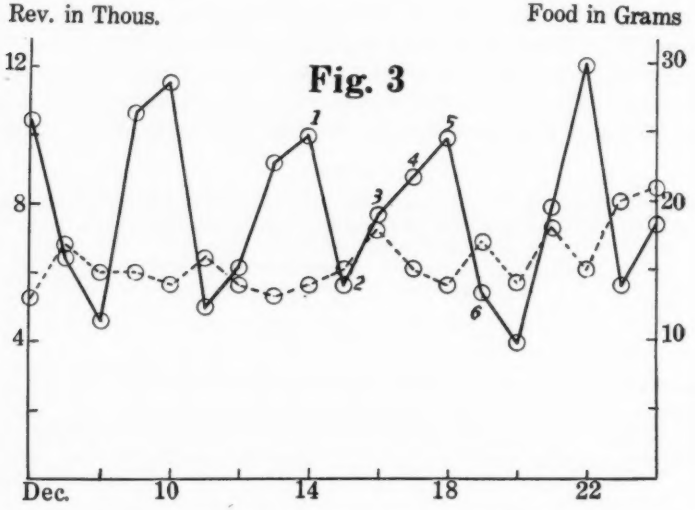


Fig. 3. Graph showing the daily activity, continuous line, and food consumption, dotted line, of a young virgin rat. F 4, at the age of 148 days. The circles represent the number of revolutions turned and the grams of food eaten during the preceding twenty-four hours. *1* and *5* indicate oestrus. Detail of the activity from *1* (Dec. 14) to *6* (Dec. 19) is shown in figure 4 A, B and C.

In many cases it was followed by a complete cessation of activity for one or more hours (fig. 6, C and D). December 15, 16, 17 and 19 of figure 4 show the distribution of the activity during the pre-oestrual, post-oestrual and dioestrual periods as indicated by *2*, *3*, *4* and *6* of figure 3. It is readily seen that the duration of vigorous activity in each of these cases was less than during oestrus. In both cases of oestrus the activity was continuous; i.e., there was not a single hour, after the activity began, during which no running was done. In the other activity periods of the oestrual cycle there were one or more hours intervening during which not a single revolution was turned. These curves show that in the young virgin there

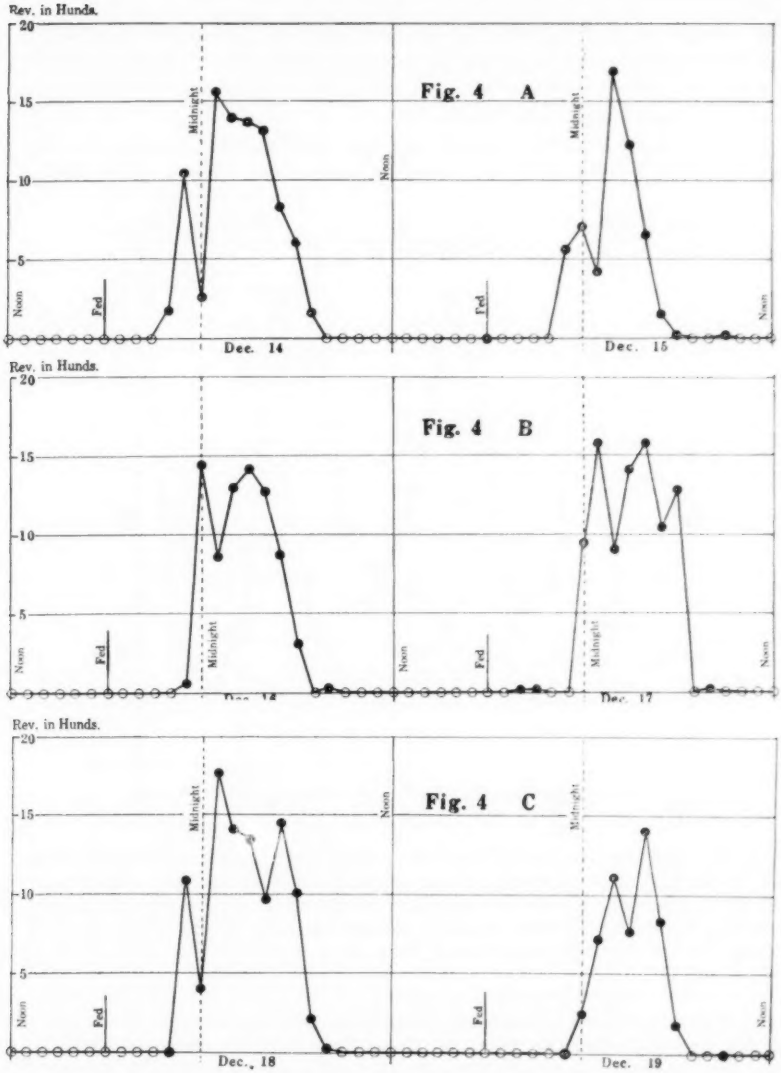


Fig. 4 A. Graph showing the hourly distribution of the activity of a young virgin rat, E 4, during oestrus, December 14, and during dioestrus, December 15 (fig. 3, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

Fig. 4 B. Showing hourly distribution of activity of same rat during dioestrus and pre-oestrus, December 16 and 17 (fig. 3, *3* and *4*).

Fig. 4 C. Showing hourly distribution of activity of same rat during oestrus, December 18, and post-oestrus, December 19, (fig. 3, *5* and *6*).

was a lengthening of the period of intensive activity and a lack of random and intermittent running during oestrus as compared to the other periods of activity in the oestrual cycle. During these days this rat rested or was inactive 58 per cent of the time.

In order to demonstrate whether age had any marked influence on the character of the activity, we have made a comparison with an older virgin

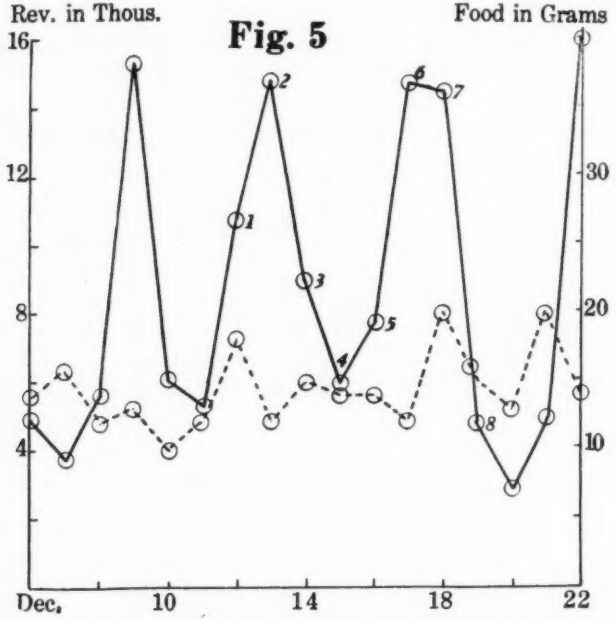


Fig. 5. Graph showing the daily activity, continuous line, and the food consumption, dotted line, of an old virgin rat, A 5, at the age of 375 days. The circles represent the number of revolutions turned and the grams of food eaten during the preceding twenty-four hours. *2* and *6* indicate oestrus. Detail of the activity through the two oestrual peaks from *1* (Dec. 12) to *8* (Dec. 19) is shown in fig. 6 A, B, C and D.

Fig. 6 A. Graph showing the hourly distribution of the activity of an old virgin rat, A 5, during pre-oestrus, December 12, and during oestrus, December 13, (fig. 5, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

Fig. 6 B. Showing the hourly distribution of activity of same rat during post-oestrus, December 14, and dioestrus, December 15 (fig. 5, *3* and *4*).

Fig. 6 C. Showing the hourly distribution of activity of same rat during pre-oestrus, December 16, and during oestrus, December 17, (fig. 5, *5* and *6*).

Fig. 6 D. Showing the hourly distribution of activity of the same rat during post-oestrus, December 18, and during dioestrus, December 19 (fig. 5, *7* and *8*).

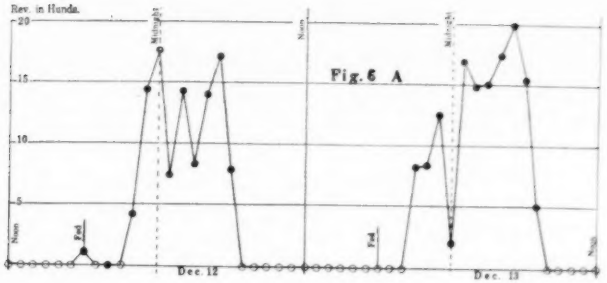


Fig. 6 A

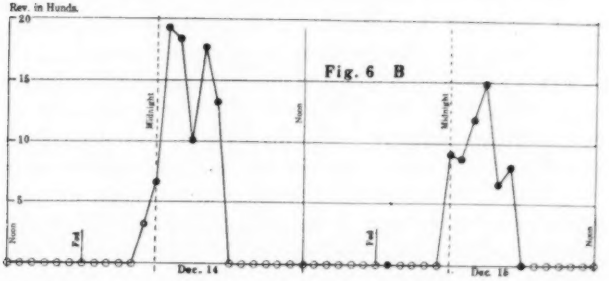


Fig. 6 B

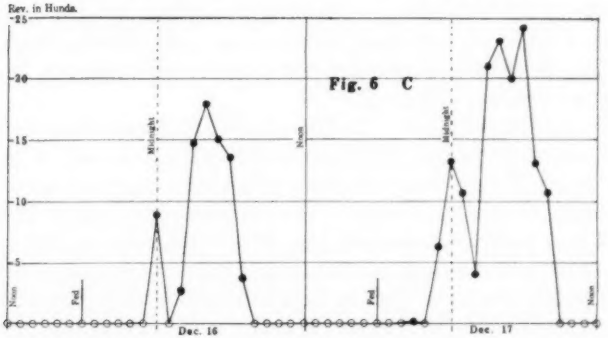


Fig. 6 C

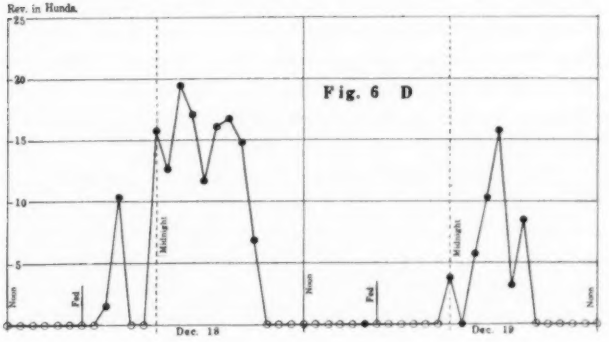


Fig. 6 D

rat, A 5, at the age of 375 days. The daily activity and food consumption are shown in figure 5. Each circle represents the total activity and food eaten during the preceding twenty-four hours. By comparing figure 5 with figure 3 it is readily seen that the average daily activity of the older animal was about 2000 revolutions more and the differences between the high and low points were much more pronounced than in the younger animal. The high points at *6* and *7* suggest that ovulation occurred some time between the two readings and that the normal four-day rhythm, found in younger animals, was becoming longer. The hourly observations of activity of the eight days marked *1* to *8* are given in graphic form in figure 6, A, B, C and D. The hourly activity during oestrus, A, December 13 and C, December 17, resembles in general that of the younger virgin. There was an initial spurt of activity and a slowing up followed by four to six hours of intensive activity. The duration of the activity was practically the same in each case. An increase in the amount of activity was noticeable. There was a decided tendency in the older animal to stop its activity more abruptly than in the younger rat. With the exception of the increased activity and a tendency of the older virgin to shorten the activity periods, the other activity periods of the oestrual cycle shown in figure 6 did not differ from those of the younger rat (fig. 4) more than would be expected owing to individual variation and age. During these observations this rat was inactive 62 per cent of the time. This was more than found in the younger virgin.

Activity of multiparous rats. In figure 7 we have shown the daily activity and food consumption of a multiparous rat, B 5, at the age of 285 days. Each circle represents the revolutions of activity and food eaten during the preceding twenty-four hours. The portions of the curve which were analyzed into hourly components (fig. 8) are indicated by the numerals *1* to *8*. This rat had given birth to two litters of ten and seven young each. The last litter was born 44 days and weaned 22 days prior to these hourly observations. The effect on activity of rearing the young had entirely disappeared. If figure 7 and figure 5 are compared, we do not notice any marked difference in the activity other than what would be expected due to individual variation and age. Figure 7 shows a typical four-day oestrual rhythm, while in the older rat, shown in figure 5, there was a breaking away from and lengthening of this rhythm as already noted. The average daily activity was also slightly greater in the multiparous rat. When the hourly observations of activity are examined, figure 8, we find that in this rat there was a pronounced tendency to lengthen the periods of activity by more or less desultory running at the beginning and end of the intensive activity period. This was true in all activity periods of the oestrual cycle. We also see that there were many hours during which the rat was entirely inactive. The intensive period shows that

there were often great reductions in the amount of the hourly activity. The two activity periods during oestrus, December 15 and December 19, show a different character. In the former the initial peak was very slight, amounting to less than 500 revolutions, while in the latter it exceeded 3000 revolutions. The total amount of activity was approximately the same in each case, but in the first instance it was spread out over fourteen hours while in the last it required but twelve hours. Each of these

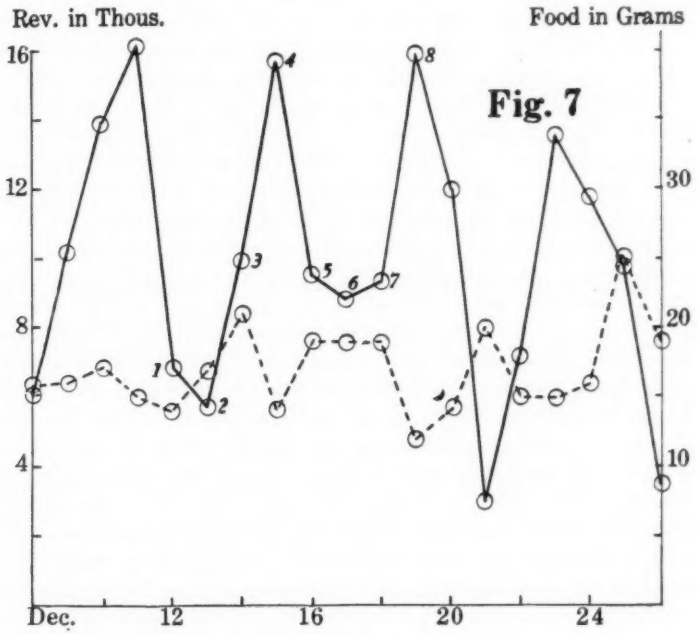


Fig. 7. Graph showing the daily activity, continuous line, and the food consumption, dotted line, of a multiparous rat, B 5, at the age of 285 days. The circles represent the number of revolutions run and the grams of food eaten during the preceding twenty-four hours. *4* and *8* indicate oestrus. Detail of the activity through the two oestrual peaks from *1* (Dec. 12) to *8* (Dec. 19) is shown in figure 8, A, B, C and D.

periods, like the other activity periods of the oestrual cycle, occupied a greater number of hours than either of the virgins already described. These results indicate that the bearing of young seems to lengthen the periods of activity and to make them more sporadic. This rat was inactive 48 per cent of the observed time.

Activity during gestation and pseudopregnancy. Figure 9 is a curve of the activity and food consumption of a multiparous rat, D 3, at the age

of 261 days, during the early days of gestation. Each circle represents the activity and food eaten for the previous twenty-four hours. The graph is a typical gestation curve in all respects. This rat had given birth to four litters and a total of 39 young. Hourly observations of the activity during the fourth and fifth days of gestation (*1* and *2*, fig. 9) are given in graphic form in figure 10. By consulting this figure it is readily seen that there were no spurts of activity and that the activity during a single hour rarely exceeded 200 revolutions. It is also noticed that the activity was spread over eighteen hours on the fourth day and seventeen hours during the fifth day of gestation. With the exception of the hour from 2 to 3 a.m., December 15, the activity was continuous in each case. A few revolutions were also run between 11 and 12 a.m., December 14. During the entire forty-eight hours there were only thirteen hours in which there was no activity. During these early days of gestation this rat was active 73 per cent of the time. This indicates an extreme condition of restlessness which was not found in any of the non-pregnant rats.

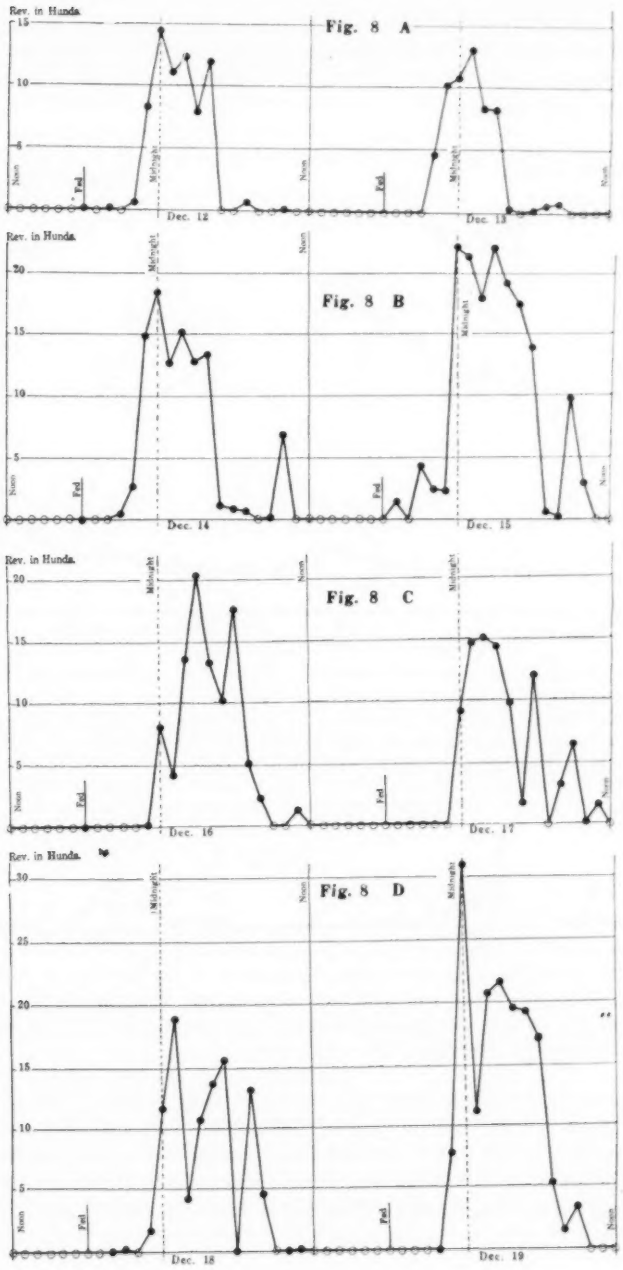
Figure 11 shows the daily activity and food consumption during the last days of gestation of a multiparous rat, C 4, at the age of 278 days. These are usual conditions and represent a typical gestation curve. This rat had previously delivered three litters, aggregating 31 young. The hourly activity during the sixteenth and seventeenth days of gestation (*1* and *2*, fig. 11) is shown in figure 12. This figure shows a marked contrast to the conditions of early pregnancy as shown in figure 10. Like the former activity curve there is a complete absence of any spurts of activity and in no instance did the hourly activity much exceed 150 revolutions. This was less than that exhibited during early pregnancy. With the exception of three scattered hours, during which only a few revolutions were turned, both the activity periods were continuous. Two of these random hours occurred at the feeding time. A disturbance at the feeding time is a very common occurrence. The two activity periods, represented by the continuous running, were short, being of only seven and six hours' duration respectively. This was in decided contrast to the activity exhibited during early pregnancy. During the early period of gestation the animal was resting only 27 per cent of the time while in late gestation it was

Fig. 8 A. Graph showing the hourly distribution of the activity of multiparous rat, B 5, during post-oestrous, December 12, and dioestrous, December 13, (fig. 7, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

Fig. 8 B. Showing the hourly distribution of the activity of the same rat during pre-oestrus, December 14, and oestrus, December 15, (fig. 7, *3* and *4*).

Fig. 8 C. Showing the hourly distribution of the activity of the same rat during post-oestrus, December 16, and dioestrus, December 17 (fig. 7, *5* and *6*).

Fig. 8 D. Showing the hourly distribution of the activity of the same rat during pre-oestrus, December 18, and during oestrus, December 19 (fig. 7, *7* and *8*).


Rev. in Hunds.
Fig. 8 A
Noon
Fed.
Midnight
Dec. 12
Dec. 13
Fig. 8 B
Dec. 14
Dec. 15
Fig. 8 C
Dec. 16
Dec. 17
Fig. 8 D
Dec. 18
Dec. 19

quiet 67 per cent of the time. The results of early gestation were thus almost completely reversed during late pregnancy. The speed of running was also markedly slower in the latter case. All the actions were much more deliberate and sluggish.

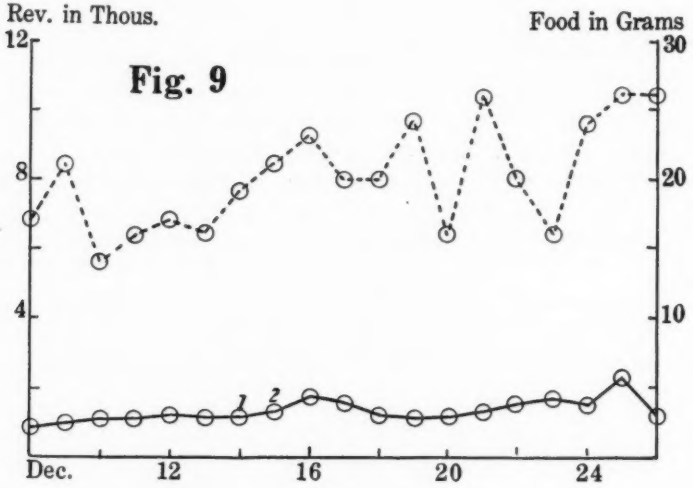


Fig. 9. Graph showing the daily activity, continuous line, and the food consumption, dotted line, during early gestation of multiparous rat, D 3, at the age of 261 days. The circles represent the number of revolutions run and the grams of food eaten during the preceding twenty-four hours. Detail of the activity of the fourth and fifth days of gestation (*1* and *2*) is shown in figure 10.

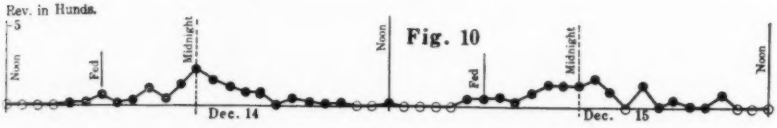


Fig. 10. Graph showing the hourly distribution of the activity of multiparous rat, D 3, during the fourth and fifth days of gestation, December 14 and 15 (fig. 9, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

In figure 13 we have shown the curves of daily activity and food consumption of rat D 1 at the age of 278 days. This rat had previously delivered two litters which averaged nine young each. Each circle represents the number of revolutions turned and the food eaten during the preceding twenty-four hours. This figure shows the results which occurred

during pseudopregnancy. The animal copulated December 10. Following this there was the marked reduction in activity and increase in the food intake characteristic of early gestation. This manifestation, however, only lasted about nine days when there began an increase in activity culminating in a typical oestrual peak fourteen days after copulation.

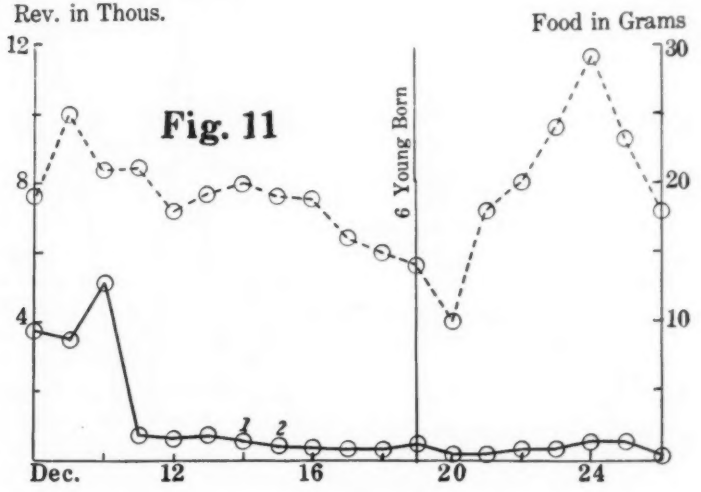


Fig. 11. Graph showing the daily activity, continuous line, and the food consumption, dotted line, during late gestation of multiparous rat, C 4, at the age of 278 days. The circles represent the number of revolutions run and the grams of food eaten during the preceding twenty-four hours. Detail of the activity of the sixteenth and seventeenth days of gestation (*1* and *2*) is shown in figure 12.

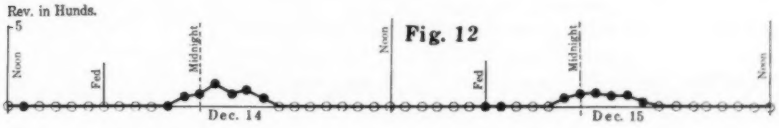


Fig. 12. Graph showing the hourly distribution of the activity of multiparous rat, C 4, during the sixteenth and seventeenth days of gestation, December 14 and 15 (fig. 11, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

Accompanying this there was the usual reduction in food eaten. We have given the hourly readings of the sixth and seventh day after coitus (*1* and *2*, fig. 13) in figure 14. This graph shows results noticeably different from either of the true gestation curves previously referred to. In each of the activity periods there was a distinct and more abrupt increase which

occurred about the same hour. This increased activity was also much greater. It is also seen that the activity was more scattered and that there was no period of continuous activity of more than four hours'

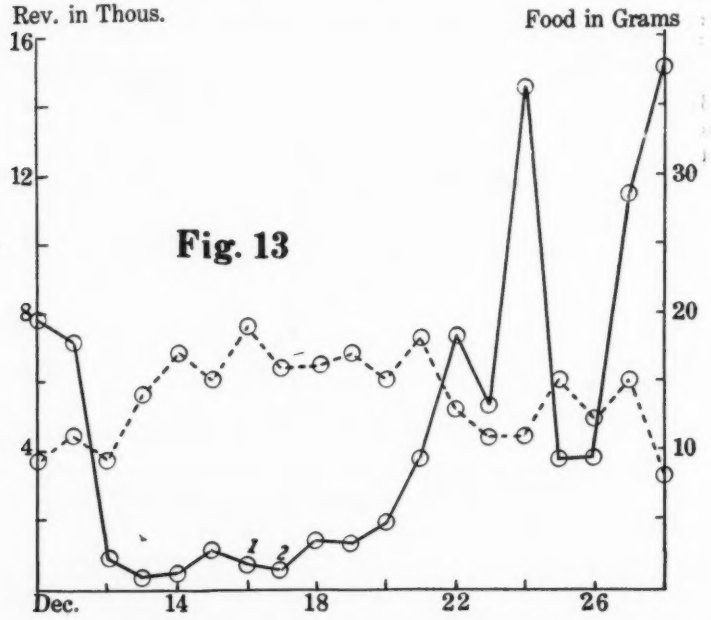


Fig. 13. Graph showing the daily activity, continuous line, and the food consumption, dotted line, during pseudopregnancy of multiparous rat, D 1, at the age of 278 days. The circles represent the number of revolutions run and the grams of food eaten during the preceding twenty-four hours. Detail of the activity of the sixth and seventh days after coitus (*1* and *2*) is shown in figure 14.

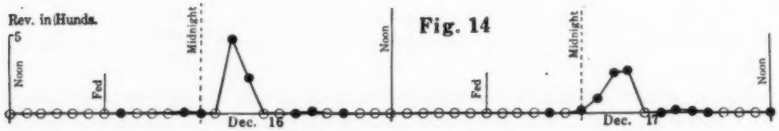


Fig. 14. Graph showing the hourly distribution of the activity of multiparous rat D 1, during the sixth and seventh days of pseudopregnancy, December 16 and 17 (fig. 13, *1* and *2*). The open circles indicate inactivity and the solid circles the amount of activity during the preceding hour.

duration. During these two days this rat was inactive 64 per cent of the time. In this respect it resembled late pregnancy, but the distribution and character of the activity were not similar.

SUMMARY

1. The albino rat is nocturnal, usually running very little during the daytime regardless of the stage of the oestrual cycle. Great variation was found in the hour of beginning the nightly run.

2. The main part of the activity occurred between the hours of 8 p.m. and 10 a.m. The maximum hourly activity usually occurred between 12 midnight and 4 a.m.

3. The duration of the different periods of activity in the oestrual cycle was nearly constant in non-pregnant rats.

4. The oestrual peak was due to augmented activity rather than to an increase in the duration of the activity period.

5. An initial spurt in activity followed by retardation just before the main nightly run was very common.

6. A fairly close resemblance was seen between the activity of the pre-oestrual and oestrual periods. A similar relation obtained between the post-oestrual and dioestrual periods.

7. Activity during oestrus was usually continuous. During other times it was often interrupted by rest of one or more hours' duration.

8. The part of the time during which the rats were inactive was: young virgins, 58 per cent; old virgins, 62 per cent; non-pregnant multiparous rat, 48 per cent; multiparous rat in early pregnancy, 27 per cent; multiparous rat in late pregnancy, 67 per cent; pseudopregnant rat, 64 per cent.

9. In all activity periods of the oestrual cycle the main part of the running was performed in the early part of the activity period.

10. The first half of the total run was accomplished earlier during post-oestrus and dioestrus than during pre-oestrus and oestrus. The descending order was: dioestrus, post-oestrus, pre-oestrus and oestrus.

11. Similarity of behavior indicates that the activating hormone exerted an influence not only during the oestrual period of activity but also to some extent during the pre-oestrual period.

12. Previous matings during the pre-oestrual period and a resemblance of the activity of this period and that of the oestrual period suggests that ovulation occurred early in the latter period or between it and the pre-oestrual period.

13. Age, the previous delivery of young, gestation and pseudopregnancy seem to have had a distinctive influence on the character of the activity.

14. A marked degree of restlessness was exhibited during early pregnancy and lassitude during late gestation.

15. Activity during pseudopregnancy differed from that of both early and late pregnancy.

BIBLIOGRAPHY

SLONAKER, J. R. 1925. This Journal, lxxi, 362.